HOSPITAL LIBRARY

VOLUME 88 NUMBER 5

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

May, 1951

The Effect of Adrenocorticotropic Hormone (ACTH) and Cortisone on Histamine Reactions and Anaphylactic Reactions in Guinea Pigs. S. Walter Landau, William A. Nelson, and Leslie N. Gay 395

Studies on Afibrinogenemia. I. Afibrinogenemia in a Patient with Septic Abortion, Acute Yellow Atrophy of the Liver and Bacteremia due to E. Coli. C. Lockard Conley, Oscar D. Ratnoff, and Robert C. Hartmann 402

Studies on Afibrinogenemia. II. The Defibrinating Effect on Dog Blood of Intravenous Injection of Thromboplastic Material. Oscar D. Ratnoff and C. Lockard Conley .. 414

Streptomycin in the Treatment of Tuberculous Pericarditis. Douglas Carroll 425

The Pathology of the Osseous Changes in Cushing's Syndrome in an Infant and in Adults. Richard H. Follis, Jr. 440

Modified One-Stage Hepatectomy in the Dog: With Some Notes on the Effect of Hepatectomy on the Coagulability and Proteolytic Activity of the Blood. Richard C. Clay and Oscar D. Ratnoff 457

"Giant Molecules" and Cholesterol in Relation to Atherosclerosis. Ancel Keys 473

Proceedings of the February 1951 Meeting of the Johns Hopkins Medical Society 484

Book Reviews 493

Books Received for Review 498

PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland

Made in United States of America

Copyright, 1951, by The Johns Hopkins Press

BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

MANAGING EDITOR, FREDERIK B. BANG

ASSOCIATE MANAGING EDITOR, H. WILLIAM SCOTT, JR.

EDITORIAL BOARD

E. Cowles Andrus
Charles R. Austrian
Kenneth C. Blanchard
Edward M. Hanrahan
John Eager Howard
Arnold R. Rich

All correspondence relating to the publication of papers should be addressed to The Editor, Bulletin of The Johns Hopkins Hospital, Baltimore-5, Md.

Books for review should be sent to The Editor at the above address.

Twenty-five reprints, without covers, of articles will be furnished to contributors free of cost. An order slip for additional reprints, with a table showing cost, is sent with proof.

Correspondence concerning business matters and all remittances should be sent to The Johns Hopkins Press, Baltimore-18, Maryland, U. S. A.

This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

New subscriptions and renewals are entered to begin with the first issue of the current year. Should any issue of the current volumes be out of print at the time the subscription order is received, the pro rata value of such numbers will be refunded.

Subscriptions should be renewed promptly. To avoid a break in your series, subscriptions should be renewed promptly. The Publisher cannot guarantee to supply back issues on belated renewals.

Subscriptions, new or renewal, may be sent to the Publishers or ordered through your agent.

Subscriptions in the British Empire except Canada may be sent to: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, London, W.C. 2.

THE JOHNS HOPKINS PRESS
BALTIMORE-18, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Post Office. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

THE EFFECT OF ADRENOCORTICOTROPIC HORMONE (ACTH) AND CORTISONE ON HISTAMINE REACTIONS AND ANAPHYLACTIC REACTIONS IN GUINEA PIGS*

S. WALTER LANDAU, WILLIAM A. NELSON AND LESLIE N. GAY†

Received for publication November 20, 1950

The effect of adrenalectomy on antibody titer was the subject of investigations as early as three and four decades ago. The results were conflicting. (Lit. see Herbert and de Vries 1). In the past few years Dougherty, White and Chase (2, 3, 4, 5, 6, 7, 8) carried out a series of studies concerning the relationship of pituitary and adrenal cortical hormone to antibody circulation. Their conclusions may be summarized as follows: Antibodies are generally present in the beta or gamma globulin fractions of the serum protein. Gamma globulin is released from lymphocytes which are disintegrating either as a result of increased secretion of adrenal cortical hormone as or a result of injection of adrenal cortical extract (ACE) or adrenocorticotropic hormone (ACTH). Therefore, these authors conclude, due to the dissolution of lymphocytes, an enhancement of circulating antibody concentration takes place whenever the amount of "pituitary-adrenal cortical hormones" (5) has been augmented in the organism.

Eisen and co-workers (9), Thatcher and co-workers (10), Herbert and de Vries (1), and Fischl and co-workers (11) confirmed the diminution of circulating lymphocytes after the injection of ACE or ACTH, but found no increase in the antibody level. Fischl and co-workers (11) suggest that the usual serological titration may be subject to large errors, and they conclude that the inference drawn by other investigators as to the relationship of hormone and lymphocytes to circulating antibodies must be in doubt. Habel and co-workers (12) likewise believe that until more critical immunological techniques and better controlled experiments are employed, the immunological evidence for

* This investigation has been aided by grants from G. D. Searle and Company and from the Houston Endowment, Incorporated.

† From the Allergy Clinic of the Department of Medicine, The Johns Hopkins Hospital.

lymphocyte production of antibodies must be considered incomplete. Ehrich and co-workers (13) moreover believe that the plasma cell, not the lymphocyte, is responsible for antibody formation.

While thus the question of the effect of ACE and ACTH on antibodies remains in the stage of controversy in the laboratory, the clinical use of ACTH and cortisone in allergic conditions has been attempted and encouraging results have been reported by a number of workers (14, 15, 16, 17, 18, 19). These clinical trials were stimulated not on the basis of the experimental studies discussed above, but mainly because of the relationship between a hypersensitivity state and the rheumatic diseases in which cortisone and ACTH had been used with conspicuous success.

In view of the suggestion that ACTH may block hypersensitivity reactions in patients, one question has assumed increased importance: On what link in the chain of such reactions may this blocking take place? With this question in mind, we carried out the following studies.

1. ACTH AND HISTAMINE REACTIONS

(a) Dale test: Two guinea pigs of 250 gm. weight received a single dose of 16 mg/kg ACTH, subcutaneously. One animal was sacrificed four hours and the other six hours after the injection. The intestinal strips were suspended in the Dale bath with the technique previously described (20). Addition of from 1 to 10 gamma histamine base to 100 cc. Tyrode solution caused vigorous contractions, as high as or higher than those of an untreated control. Two other animals were injected with 2 mg/kg ACTH every six hours for four days, a total dose of 30 mg/kg. Four and six hours, respectively, after the last s.c. injection of ACTH, the Dale test was performed. The gut of both animals again responded to doses of histamine base ranging from 0.1 to 10 gamma per 100 cc. with contractions as high as or higher than those of the controls. The ACTH used in these experiments was supplied in the fall of 1949 by Armour and Company through Dr. A. M. Harvey.

(b) Intact animal: Four guinea pigs were treated with 6 mg/kg ACTH s.c. every six hours for four days, a total dose of 96 mg/kg. Four hours after the last injection of ACTH, histamine acid phosphate (Lilly), the equivalent of 0.5 mg/kg histamine base, was injected into the jugular vein. We had consistently found this to be the minimal lethal dose, killing at least 90% of the animals. The four animals died within two minutes, and autopsy showed the typical findings of histamine death.

The ACTH used in this series was supplied by Searle and Company through the

courtesy of Dr. Irwin Winter. It was a batch marked "not sterile." No attempt was made to sterilize the drug, but it was handled and dissolved with sterile precautions. In the biological assay, the potency of this batch was reported to be from 1/10 to 1/15 of Armour Standard*. In clinical trials, according to information received from Searle and Company, it had proved potent.

2. CORTISONE AND HISTAMINE INTOXICATION

Nine guinea pigs were treated subcutaneously with 5 mg/kg cortisone (Merck) twice daily, for four days, a total dose of 40 mg/kg. Four hours after the last injection, one lethal dose of histamine was given intravenously. Eight animals (approximately 90%) died, and dissection exhibited the picture of histamine death.

3. ACTH AND ANAPHYLACTIC REACTIONS

(a) Dale tests: Intestinal strips from two actively sensitized guinea pigs† were suspended in Tyrode solution, 30 and 90 minutes, respectively, after the injection of a single dose of 10 mg/kg ACTH. 0.5 cc. sheep serum added to the bath caused contractions in both cases as high as or higher than those in untreated controls.

Two other guinea pigs were injected intraperitoneally with 2 cc. antihuman rabbit serum. On the same day, treatment was started with 2.5 mg/kg ACTH injected every six hours. This was continued for four days. Four and six hours, respectively, after the last dose, the animals were sacrificed and intestinal strips suspended. Human serum (0.5 cc.) caused vigorous contractions in both cases.

(b) Intact animal: Three guinea pigs actively sensitized with sheep serum received a single dose of 2 mg/kg ACTH. The challenging dose,‡ administered 30 minutes later, caused anaphylactic death in the three animals. The result was the same in three other sensitized guinea pigs that had received a single dose of 10 mg/kg ACTH preceding the shocking dose.

ACTH was next given to nine animals every six hours for four days: 2.5 mg/kg per dose to 3 guinea pigs sensitized with a highly potent antihuman rabbit serum;§ 4 mg/kg per dose to 4 actively sensitized guinea pigs; 6 mg/kg to 2 other actively sensitized guinea pigs. After the challenging dose given four to six hours after the

---

* All data on biological assays in this paper were supplied by the companies who furnished the drugs.

† Active sensitization was carried out in this and all other experiments with two intraperitoneal doses of 0.5 cc. fresh sheep serum administered four or five days apart.

‡ All actively sensitized guinea pigs were challenged with 0.25 or 0.5 cc. sheep serum 20 to 24 days after the second sensitizing dose.

§ Passive sensitization was carried out with 2 cc. serum i.p. on the first day of treatment with ACTH. The challenging dose was 1 cc. human serum.

last dose of ACTH, severe shock symptoms developed in all these animals. One of the three passively sensitized and three of the six actively sensitized guinea pigs died. The three groups were too small (due to the scarcity of ACTH at that time) to allow evaluation of the mortality.

In a third group of animals, antihuman rabbit serum of only moderate sensitizing power was used: the challenging dose caused mild symptoms, but no deaths in three controls. In four guinea pigs sensitized with this serum and treated with 4 mg/kg ACTH every six hours for four days, the challenging dose produced shock symptoms which were in no way different from those of the controls.

The ACTH used in all the anaphylaxis experiments discussed so far was sterile powder obtained from Searle and Company. Its potency was one-half of that of Armour Standard. The drug belonged to the same batch that was used in the rabbit experiments of Berthrong, Rich and Griffith (21).

After the conclusion of these studies, we obtained a new ACTH preparation from Armour. It was rated to have a potency of 160% of Armour Standard. Actively sensitized guinea pigs were injected with 1.25 mg/kg of this preparation every six hours for four days. Four hours after the last dose of ACTH the injection of the challenging dose of sheep serum resulted in fatal anaphylactic shock within three minutes in six of the seven animals. Of the nine controls, eight died.

4. CORTISONE AND ANAPHYLACTIC REACTIONS

Nineteen guinea pigs were sensitized with sheep serum. Three weeks later, ten of the animals were treated with 5 mg/kg cortisone (Merck) twice a day for four days. Four hours after the last dose of cortisone, the challenging dose was injected. Fatal shock occurred in six animals; the four that survived had typical shock symptoms. Mortality in the untreated controls was approximately the same: six of the nine controls died from the re-injection of sheep serum.

DISCUSSION

Our studies demonstrate that in the guinea pig administration of ACTH or of cortisone in the doses used does not prevent the union of antigen and antibody, nor interfere with the effects of such union, nor does it protect against histamine reactions.

Leger and co-workers (22), 1948, observed no protective effect from a single dose of ACTH administered six to eight hours before the re-injection of the antigen into actively sensitized guinea pigs. Recently, Friedlaender and Friedlaender (23) using likewise a single dose of ACTH failed to obtain any protection in guinea pigs against histamine aerosol or against anaphylactic shock.

We extended the observations to the isolated guinea pig gut, and we used both actively and passively sensitized guinea pigs. Furthermore,

in addition to testing by single doses, we treated the animals for four days, and we used cortisone as well as ACTH. We, too, arrived at negative results.

The doses that we used varied according to the different potency of the preparations. The daily doses on a comparable weight and potency basis were at least equal to, in many instances higher than, those successfully used in humans (compare e.g. Carey and coworkers) (24).

It should be stressed that ACTH and cortisone were not only unable to counteract fatal doses of histamine or antigen, but that these compounds were also unable to suppress the effects of smaller doses. Such inefficacy was seen in the Dale tests with histamine and in those guinea pigs which received only mildly potent antihuman rabbit serum. This is in agreement with Fischl (25) who, employing Kabat's quantitative technique for the induction of anaphylaxis, found no alteration from normal susceptibility to anaphylactic shock in intact guinea pigs treated with ACTH.

SUMMARY

1. ACTH given in a single dose or every six hours for four days did not prevent histamine effects on the intestinal strip of guinea pigs. ACTH or cortisone given every six hours for four days did not prevent death from one lethal dose of histamine.

2. ACTH given in a single dose or every six hours for four days did not prevent anaphylactic reactions in vivo or in vitro in actively or passively sensitized guinea pigs. ACTH or cortisone given every six hours for four days did not decrease the mortality from anaphylactic shock in actively sensitized guinea pigs.

BIBLIOGRAPHY

1. Herbert, P. H., and de Vries, J. A.: The Administration of Adrenocorticotrophic Hormone to Normal Human Subjects. The Effect on the Leucocytes in the Blood and on Circulating Antibody Levels. Endocrinology **44:** 259, 1949
2. Dougherty, T. F., White, A., and Chase, J. H.: Relationship of the Effects of Adrenal Cortical Secretion on Lymphoid Tissue and on Antibody Titer, Proc. Soc. Exper. Biol. & Med., **56:** 28, 1944
3. Dougherty, T. F., Chase, J. H., and White, A.: Demonstration of Antibodies in Lymphocytes: Proc. Soc. Exper. Biol. & Med., **57:** 295, 1944
4. Dougherty, T. F., Chase, J. H., and White, A.: Pituitary-Adrenal Control

of Antibody Release from Lymphocytes. Explanation of Anamnestic Response: Proc. Soc. Exper. Biol. & Med., **58:** 135, 1945

5. Chase, J. H., White, A., and Dougherty, T. F.: Enhancement of Circulating Antibody Concentration by Adrenal Cortical Hormone, J. Immunol., **52:** 101, 1946
6. White, A., and Dougherty, T. F.: Role of Lymphocytes in Normal and Immune Globulin Production and Mode of Release of Globulin from Lymphocytes. Ann. New York Acad. Sc., **46:** 859, 1946.
7. White, A., and Dougherty, T. F.: Effect of Prolonged Stimulation of the Adrenal Cortex and of Adrenalectomy on the Numbers of Circulating Erythrocytes and Lymphocytes. Endocrinology **36:** 16, 1945
8. White, A., and Dougherty, T. F.: The Pituitary Adrenotrophic Hormone Control of the Rate of Release of Serum Globulins from Lymphoid Tissue. Endocrinology **36:** 207, 1945
9. Eisen, H. N., Mayer, M. M., Moore, D. H., Tarr, R. R., and Stoerk, H. C.: Failure of Adrenal Cortical Activity to Influence Circulating Antibodies and Gamma Globulin. Proc. Soc. Exper. Biol. & Med. **65:** 301, 1947
10. Thatcher, J. S., Houghton, C., and Ziegler, C. H.: Effect of Adrenalectomy and Adrenal Cortical Hormone upon the Formation of Antibodies. Endocrinology **43:** 440, 1948
11. Fischl, E., LeMay, M., and Kabat, E.: The Effect of Adrenocorticotrophic Hormone and X-ray on the Amount of Circulating Antibody. J. Immunol. **61:** 89, 1949
12. Habel, K., Endicott, K. M., Bell, F., and Spear, F. J.: Immunological Evidence on the Role of the Lymphocyte in Antibody Formation. J. Immunol. **61:** 131, 1949
13. Ehrich, E., Drabkin, D. L., and Forman, C.: Nuclei Acids and the Production of Antibody by Plasma Cells. J. Exper. Med. **90:** 157, 1949
14. Bordley, J. E., Carey, R. A., Harvey, A. M., Howard, J. E., Kattus, A. A., Newman, E. V., and Winkenwerder, W. L.: Preliminary Observations on the Effect of Adrenocorticotropic Hormone (ACTH) in Allergic Diseases. Bull. Johns Hopk. Hosp. **85:** 396, 1949
15. Proceedings of the First Clinical ACTH Conference, ed. by Mote, J. R., Philadelphia, 1950
16. Randolph, T. G., and Rollins, J. P.: Adrenocorticotropic Hormone (ACTH). Its Effect in Bronchial Asthma and Ragweed Hay Fever. Ann. Allergy **8:** 149, 1950
17. Rose, B., Pare, J. A. P., Pump, K., and Stanford, R. L.: Preliminary Report on Adrenocorticotropic Hormone (ACTH) in Asthma. The Canad. Med. Assn. Journ. **62:** 6, 1950
18. Carryer, H. M., Koelsche, G. A., Prickman, L. E., Mayrum, C. K., Lake, C. F., and Williams, H. L.: The Effect of Cortisone on Bronchial Asthma and Hay Fever Occurring in Subjects Sensitive to Ragweed Pollen. J. Allergy **21:** 282, 1950

19. RANDOLPH, T. G., AND ROLLINS, J. P.: The Effect of Cortisone on Bronchial Asthma. J. Allergy **21:** 288, 1950
20. LANDAU, S. W., AND GAY, L. N.: Comparative Study of Antihistamine Substances. 1. Introduction and Dale Experiments. Bull. Johns Hopk. Hosp. **83:** 330, 1948
21. BERTHRONG, M., RICH, A. R., AND GRIFFITH, P. C.: A Study of the Effect of Adrenocorticotrophic Hormone (ACTH) upon the Experimental Cardiovascular Lesions Produced by Anaphylactic Hypersensitivity. Bull. Johns Hopkins Hosp. **85:** 131, 1950
22. LEGER, J., LEITH, W., AND ROSE, B.: Effect of Adrenocorticotrophic Hormone on Anaphylaxis in Guinea Pigs. Proc. Soc. Exper. Biol. & Med. **69:** 465, 1948
23. FRIEDLAENDER, S., AND FRIEDLAENDER, A. S.: The Effect of Pituitary Adrenocorticotrophic Hormone (ACTH) on Histamine Intoxication and Anaphylaxis in the Guinea Pig. J. Allergy **21:** 303, 1950
24. CAREY, R. A., HARVEY, A. M., HOWARD, J. E., AND WINKENWERDER, W. L.: The Effect of Adrenocorticotrophic Hormone (ACTH) and Cortisone on the Course of Chronic Bronchial Asthma. Bull. Johns Hopkins Hosp. **87:** 387, 1950
25. FISCHL, E. E.: The Relationship of Adrenal-Cortical Activity to Immune Responses. Bull. New York Academy of Medicine **26:** 255, 1950

STUDIES ON AFIBRINOGENEMIA

I. Afibrinogenemia in a Patient with Septic Abortion, Acute Yellow Atrophy of the Liver and Bacteremia Due to E. Coli

C. LOCKARD CONLEY, OSCAR D. RATNOFF AND ROBERT C. HARTMANN

Department of Medicine, The Johns Hopkins University and Hospital

Received for publication December 11, 1950

In rare instances, an absence of detectable fibrinogen has been observed in patients with certain disorders. In some of these patients the lack of fibrinogen was apparently the result of a congenital defect. In others, the absence of fibrinogen was associated with severe hepatic damage, with certain complications of pregnancy, or with metastatic carcinoma.

In the congenital cases, the absence of fibrinogen is presumably attributable to defective production of fibrinogen. In other conditions, the pathogenesis of afibrinogenemia has been less clear. It is generally believed that the afibrinogenemia associated with disease of the liver is the result of impaired production of fibrinogen. However, in some patients with hepatic disease, the plasma has fibrinolytic properties. It is not known whether afibrinogenemia in these and other patients may result from the activity of the plasma's intrinsic proteolytic enzymes. It is conceivable that in some disorders, afibrinogenemia may result from intravascular clotting of all the available fibrinogen.

In the present report, certain observations are described relating to these possible mechanisms for the production of afibrinogenemia. A patient with afibrinogenemia was studied. This patient had acute yellow atrophy of the liver and a bacteremia of *E. coli* following an abortion. Although no fibrinogen could be detected in the patient's blood, the prothrombin concentration was not greatly reduced. Studies were

This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service and was in part carried out under contract with the Office of Naval Research, United States Navy.

performed to determine whether the absence of detectable fibrinogen could be attributed to a lytic process, or to release of thromboplastic material into the blood from the infected uterus, or whether it was more likely attributable to the hepatic derangement. The results of these investigations indicated that the absence of fibrinogen could not be ascribed to excessive lysis, either by the patient's plasma proteolytic enzyme or by the infecting organism, a species known to be fibrinolytic *in vitro* (1). In the accompanying paper, experiments are described in which afibrinogenemia was produced by intravenous injection of thromboplastic material (2). The amount of thromboplastin needed to produce this effect was so great that this mechanism seems unlikely as a cause of clinical afibrinogenemia. Presumably, the fibrinogen deficiency in this patient was the result of diminished production of this protein.

Case Report. I. B. (J.H.H. #380553), a 29 year old colored housewife, was admitted to the hospital in coma. The patient was apparently in good health until six days before admission, when she complained of a frontal headache which persisted. For two days before admission, she was said to feel feverish and chilly. On the morning of admission, she "shook all over" and thereafter became unresponsive. It was learned that her menstrual period was about 10 days overdue. Apparently for this reason, the patient had ingested an unknown amount of "Watkins liniment", sometime during the week prior to admission. This liniment contains camphor, oil of spruce, safrol, capsicum and alcohol.

At the time of admission, the patient was critically ill and comatose. Her rectal temperature was 105°F., the respiratory rate was 30 per minute, and the respirations were shallow. The pulse could not be obtained at the wrist, but the heart rate was 120 per minute. Although the blood pressure was difficult to obtain, the systolic pressure was thought to be 50 mm. Hg. The skin of the extremities was cold and moist, but that of the trunk was hot. A petechia was observed in the left conjunctiva. The sclerae were slightly icteric, and the mucous membranes pale. There was a small amount of dried blood in the nose, and the gums were bleeding. On pelvic examination, the uterus was found to be approximately one and a half times normal size. It was firm and freely movable. There was a slight bloody discharge from the cervix. The remainder of the physical examination was not remarkable.

The serologic test for syphilis was negative. The hematocrit was 32 per cent, the hemoglobin 9.8 grams per 100 ml. The white blood cell count was 11,900 per cmm. On the blood smear, there were 1 per cent myelocytes, 15 per cent juvenile neutrophils, 64 per cent polymorphonuclear neutrophils, 1 per cent eosinophils, 13 per cent lymphocytes and 6 per cent monocytes. There were 2 nucleated red cells per

100 white cells. The platelet count was 30,000 per cmm. The sedimentation rate was 21 mm. in one hour, uncorrected (Wintrobe). The icterus index was 20. A urine specimen obtained by catheterization contained a faint trace of albumin, but no red blood cells. The Wallace and Diamond test for urobilinogen was positive in urine diluted one to 80, but bile was not detected. A stool specimen was grossly bloody. The spinal fluid was normal. Two blood cultures were positive for *E. coli* var. *communior*, the organism being found in liquid media but not in direct pour plates. The same organism was obtained from the uterine cervix.

The patient's blood failed to clot in glass test tubes. The bleeding time from the ear was greater than one hour. Petechiae and ecchymoses appeared after application of a tourniquet to the arm, and hematomas developed at the sites of venepunctures. Large amounts of penicillin and vitamin K were administered, and the patient was transfused with 1500 ml. of citrated bank blood. She was also given 550 ml. of fresh blood by direct transfusion, using syringes and needles treated with silicone. After 1000 ml. of blood had been injected, bleeding from from the gums and from the various puncture wounds was observed to cease. However, 12 hours after her admission to the hospital, the patient died.

At autopsy, there was evidence that the patient had had an incomplete abortion and that the endometrium was infected and necrotic. There was thrombophlebitis of the right ovarian vein. The liver weighed 1400 grams. Grossly and microscopically it showed evidence of severe acute yellow atrophy. The kidneys showed the changes of severe nephrosis, with extensive destruction of the tubular epithelium. Necrosis and severe degenerative changes were present in the adrenals. The lungs were congested and edematous. There were extensive visceral hemorrhages but no intracranial bleeding was demonstrated.

EXPERIMENTAL DATA

Studies on the nature of the clotting defect

Venous blood drawn from the patient at the time of admission did not clot in glass tubes within 24 hours. A number of experiments were performed to determine the nature of the clotting defect. Oxalated plasma, prepared by the addition of one part of 0.1M sodium oxalate to 9 parts of blood, failed to clot when recalcified in the presence of rabbit brain thromboplastin. The addition of a large amount of powdered bovine thrombin (Upjohn) both to native plasma and to oxalated plasma did not induce coagulation. These observations suggested either that fibrinogen was absent from the patient's blood or that the fibrinogen present could not be clotted by thrombin. For this reason, the effect of the addition of human and bovine fibrinogen to the patient's plasma was tested.

Prothrombin-free plasma prepared from hemophilic blood was used

as a source of human fibrinogen. Oxalated hemophilic plasma was incubated with powdered barium sulfate at 37°C. for 10 minutes. The supernatant plasma failed to clot within one hour upon recalcification in the presence of rabbit brain thromboplastin, indicating that the prothrombin had been completely removed. A mixture of 0.1 ml. each of the patient's native plasma, the prothrombin-free oxalated plasma, rabbit brain thromboplastin, and 0.025M calcium chloride resulted in clotting after 15 seconds at 37°C.

This experiment demonstrated that clotting occurred when prothrombin-free plasma containing human fibrinogen was added to the patient's plasma.

TABLE I

Effect of Patient's Native Plasma on the Clotting Time of Normal Blood

Clotting time (at 37°C) of 1 ml. portions of normal blood to which was added the patient's plasma in the amounts indicated

0.2 ML BUFFER	0.5 ML. BUFFER	0.2 ML. PT.'S PLASMA	0.5 ML PT'S PLASMA
min.	min.	min.	min.
12	16	18	18
17	20	23	52
20	23	23	70

One-tenth ml. of the patient's oxalated plasma was mixed with 0.1 ml. of 0.5 per cent bovine fibrinogen;[1] 0.1 ml. of rabbit brain thromboplastin, and 0.1 ml. of 0.025M calcium chloride. In duplicate experiments at 37°C., clots appeared in 42 and 46 seconds.

The clotting defect in this patient, then, was to a large extent correctable by the addition of a preparation of bovine fibrinogen.

These observations demonstrated that the failure of the patient's blood to clot was attributable to an absence of fibrinogen. Further studies were performed to determine whether other abnormalities of the clotting mechanism were present. Experiments were carried out to determine whether the patient's plasma would retard the coagulation of normal blood. The patient's plasma, obtained without the addition of an anticoagulant, was mixed with freshly drawn normal blood and the clotting time observed. The data of this experiment are recorded in Table I. These data indicated that the patient's plasma delayed the coagulation of normal blood.

[1] Bovine fibrinogen was obtained through the courtesy of Dr. W. H. Seegers.

The nature of the clot-inhibitory activity of the patient's plasma was investigated. Normal prothrombin-free plasma was clotted by thrombin in various concentrations in the presence of the patient's oxalated plasma. Control experiments were performed by substituting normal oxalated plasma for that of the patient. The results of these experiments are shown in Table II. It is apparent that the patient's oxalated plasma exerted an inhibitory effect upon the clotting action of thrombin.

TABLE II

Effect of Patient's Oxalated Plasma on the Clotting Activity of Thrombin

		CLOTTING TIME (SEC.) UPON ADDITION OF 0.1 ML. OF THROMBIN SOLUTION		
		Concentration of Thrombin (units/ml.)		
		1.0	1.5	10.0
		sec.	sec.	sec.
Patient's oxalated plasma	0.1 ml.	720	720	25
Normal prothrombin-free plasma†	0.1 ml.			
Normal oxalated plasma	0.1 ml.	120, 105*	110, 70*	10, 12*
Normal prothrombin-free plasma†	0.1 ml.			

* Duplicate experiments.

† Oxalated normal plasma was rendered prothrombin-free by adsorption on barium sulfate.

An adequate assay of the concentration of prothrombin and of the prothrombin conversion accelerator factor in the patient's plasma was not possible. It seemed unlikely, however, that great deficiency of these substances was present. As has already been described, the clotting time of a mixture of the patient's plasma, fibrinogen, thromboplastin, and calcium was not remarkably prolonged.

In summary, then, the patient had a severe hemorrhagic disorder associated with thrombocytopenia, afibrinogenemia, and a circulating inhibitor of thrombic activity.

Studies on the cause of the afibrinogenemia

Fibrinolytic Activity of the Patient's Plasma

Plasma contains a proteolytic enzyme system capable under suitable conditions of digesting fibrinogen (3). Excessive proteolytic activity of plasma has been thought to be an occasional cause of acquired afi-

brinogenemia. For this reason, clots formed in the presence of the patient's plasma were observed for the occurrence of fibrinolysis. One-tenth ml. of the patient's native plasma was mixed with 0.1 ml. of 0.5 per cent bovine fibrinogen and 0.1 ml. rabbit brain thromboplastin. The mixture, which clotted in 40 seconds, was incubated at 38°C. for 72 hours. During this period of observation, no evidence of fibrinolysis was observed. Furthermore, fibrinolysis was not observed within 72 hours when 1 ml. of normal blood was incubated in triplicate at 37°C. with 0.5 ml. of the patient's plasma. Goodpasture (4) demonstrated that even in cases where fibrinolysis occurred in as short a time as $3\frac{1}{2}$ hours after clotting, appreciable amounts of fibrinogen were present in the patient's blood. For this reason, it appears unlikely that the afibrinogenemia in the patient under study was the result of an increase in the intrinsic fibrinolytic activity of her plasma.

Fibrinolytic Activity of E. coli var. communior

Cultures of the patient's blood demonstrated that a bacteremia due to *E. coli* var. ***communior*** was present. It seemed possible that the afibrinogenemia may have been the result of the presence of this organism. Zinsser and Williams (1) have demonstrated that certain strains of *E. coli* are capable of lysing fibrin clots. For this reason, the fibrinolytic activity of the organism cultured from the patient's blood was tested.

A 6 hour sub-culture in infusion broth of the organism obtained from the patient's blood was serially diluted in 10 fold steps with infusion broth. One-tenth ml. of each dilution of the culture was mixed with 0.5 ml. of sterile oxalated normal human plasma, and 0.1 ml. of sterile 0.05M calcium chloride was added. The tubes were capped using sterile technique, and were incubated at 37°C. for 48 hours. The clots, which formed promptly in all of the tubes, were observed for evidence of lysis.

At the same time, 0.1 ml. of each dilution of the cultures of *E. coli* was mixed with 0.5 ml. of a solution of 0.5% bovine fibrinogen in barbital buffer. This mixture was then clotted with 0.1 ml. of a solution of bovine thrombin containing 10 u/ml. It was not possible to sterilize the solutions of fibrinogen and thrombin. However, the clot formed by a mixture of 0.5 ml. fibrinogen, 0.1 ml. sterile infusion

broth, and 0.1 ml. thrombin did not lyse during the period of 48 hours at 37°C.

In the experiments described, cultures of *E. coli* lysed the clots formed from human plasma and from bovine fibrinogen (Table III). In each case lysis began at the upper surface of the clot and appeared to progress downward. Fibrinolysis occurred with much higher dilutions of the organism in the clots formed from bovine fibrinogen than in those formed from oxalated plasma, perhaps because of an antibacterial effect of plasma.

Studies were also carried out to determine whether the organism could lyse fibrinogen. One-tenth ml. portions of each dilution of culture

TABLE III

Lytic Effect of Cultures of E coli var. communior on Clots Formed from Normal Human Plasma and from Bovine Fibrinogen

Approximate degree of fibrinolysis after 48 hr. (per cent)

	CONCENTRATION OF CULTURE OF E. COLI VAR. COMMUNIOR						
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	0
Human Plasma	90	90	0	0	0	0	0
Bovine Fibrinogen	100	100	100	100	100	75	0

of *E. coli* used in the preceding experiment were incubated for 48 hours at 37°C. with 0.5 ml. of a 0.5% solution of bovine fibrinogen. At the end of that time 0.1 ml. of a solution of thrombin containing 10 u/ml. was added to each tube. A clot appeared within 10 seconds in the control tube containing fibrinogen and sterile infusion broth. No clots were seen in any of the tubes to which the organism had been added. The results of this experiment suggest that the strain of *E. coli* var. *communior* studied was capable of digesting fibrinogen as well as fibrin.

Because the patient's blood showed increased thrombin-inhibitory activity, experiments were carried out to determine whether the bacterial organism might have been responsible for this phenomenon.

One-tenth ml. of a 4 hour sub-culture in infusion broth of the organism was incubated at 37°C. with 0.5 ml. of sterile oxalated normal human plasma. Other 0.5 ml. portions of the same plasma were incubated alone and with sterile infusion broth. After 24 hours, 0.1 ml. of pooled normal oxalated plasma was added to 0.1 ml. portions of each

of the incubated plasmas, and each mixture was then clotted by the addition of 0.1 ml. of a solution of bovine thrombin.

No appreciable thrombin-inhibitory activity was observed in plasma which had been incubated with the strain of *E. coli* under study (Table IV).

An attempt was made to reproduce the patient's hemorrhagic disorder by experimental infection in the rabbit with the organism derived from the patient's blood. One rabbit was given an overwhelming infection by the intraperitoneal injection of this organism. Twenty-five ml. of a 13 hour broth culture were centrifuged, the supernatant fluid discarded, and the organism washed with sodium chloride solution and

TABLE IV

Thrombin inhibitory activity of plasma incubated with E. coli var. communior

TEST SUBSTANCE	CLOTTING TIME*
	sec.
Normal oxalated plasma	43, 40
Normal oxalated plasma plus sterile infusion broth	29, 27
Normal oxalated plasma plus culture of E. coli var. communior	35, 40

* In duplicate, 0.1 ml. of normal pooled plasma, 0.1 ml. of the test substance indicated, and 0.1 ml. of solution of bovine thrombin.

re-centrifuged. The organisms were then suspended in 5 ml. sodium chloride solution and injected. Five hours later the rabbit died. The concentration of fibrinogen in its plasma prior to the injection was 445 mgm. per 100 ml., and at the time of death was 400 mgm. per 100 ml. A second rabbit was injected intravenously with the washed culture of *E. coli* on each of 4 successive days. On the first and second days, the rabbit received 0.1 ml. of washed organisms which had been concentrated 10 times. On the third and fourth days, 0.4 ml. of this preparation were injected. On the sixth day the rabbit was found dead. At autopsy, clotted blood was found in the heart. The plasma fibrinogen was 219 mgm. per 100 ml. before the first injection, and on successive days thereafter was 195, 537, and 1015 mgm. per 100 ml. Daily blood cultures were positive for *E. coli*, and the specimen with the highest concentration of fibrinogen contained many thousands of organisms per ml.

Thus, no evidence was obtained that experimental infection with the *E. coli* isolated from the patient was capable of producing afibrinogenemia.

DISCUSSION

Surprisingly few instances of afibrinogenemia have been reported. Rarely, this defect may occur congenitally. Favre-Gilly (5) was able to collect 8 cases of congenital afibrinogenemia and other cases have been reported by Henderson and his associates (6), by Corbertt (7), and by Diamond (8). This disorder is discovered early in life, there is frequently a history of consanguinity, and no evidence of underlying disease is present. In two of the reported cases there was also severe thrombocytopenia. The pathogenesis of the afibrinogenemia has been thought to be an absence of production of fibrinogen. When one patient with congenital afibrinogenemia was transfused with normal plasma, appreciable amounts of fibrinogen were detectable after 96 hours (9). In view of this, it is unlikely that congenital afibrinogenemia is due to excessive destruction or removal of fibrinogen from the blood stream.

A decrease in the concentration of plasma fibrinogen has often been noted in patients with hepatic disease (10). However, the complete absence of fibrinogen from plasma is apparently exceedingly rare in these cases. Risak (11) described a 21 year old male in whom afibrinogenemia was associated with acute yellow atrophy of the liver following severe burns. The platelet count was 84,000. Opitz and Silberberg (12) reported a case of fatal miliary tuberculosis with extensive hepatic involvement in a child. The blood of this patient was said to contain no fibrinogen. However, the only specimen examined was obtained *post mortem*. The patient described in the present report had acute yellow atrophy of the liver, and the absence of fibrinogen was presumably the result of hepatic damage. It is of interest that afibrinogenemia occurred at a time when the prothrombin concentration was not markedly reduced.

The decreased concentration of fibrinogen in the plasma of patients with hepatic disease is probably attributable to a decrease in its production. Experimentally, hypofibrinogenemia has been produced by the administration of hepatotoxic drugs (13) or by excluding the liver from the circulation (14). In certain patients with hepatic disease,

rapid fibrinolysis is demonstrable *in vitro* (4). It is conceivable, therefore, that in some instances of hepatic disease, hypofibrinogenemia may be due to destruction of this protein by the intrinsic proteolytic activity of plasma. In the patient under study, however, it was not possible to demonstrate excessive fibrinolytic activity when her plasma was clotted by the addition of either bovine fibrinogen or normal human plasma.

A bacteremia of *E. coli* var. *communior* was present in the patient. This strain of *E. coli* was capable of digesting both fibrinogen and fibrin *in vitro*. However, this fibrinolytic activity was observed only in heavy growths of the organism and could not be demonstrated in experimental infection in rabbits. It appears unlikely, therefore, that the afibrinogenemia in the patient was the result of the lytic activity of the bacterium.

Unaccountable afibrinogenemia has also been reported in a patient with carcinoma of the prostate with extensive metastases to bone (15). In several patients with an obscure hemorrhagic disorder in pregnancy, fibrinogen was absent from the plasma (16, 17). Moloney and his associates (16) suggested that some of these cases might result from excessive fibrinolytic activity of the patient's plasma, but no cases have been reported in which absence of fibrinogen was convincingly shown to be the result of excessive lysis. Another possibility is that intravascular clotting might occur and thus remove the circulating fibrinogen. In the accompanying paper, experiments will be described in which the intravenous injection of thromboplastic substance rendered the blood incoagulable, presumably by inducing the conversion of fibrinogen to fibrin (2). However, the amount of thromboplastic material needed was large, and it seems unlikely that in the cases reported clinical afibrinogenemia was produced by this mechanism.

In addition to the fibrinogen defect, the blood of the patient under study displayed other abnormalities of the coagulation mechanism. Thrombocytopenia was present. The concentrations of prothrombin and accelerator globulin were apparently not greatly altered, although quantitative assays were not performed. The patient's native plasma delayed the coagulation of normal blood. It was possible to demonstrate the presence in the patient's plasma of thrombin-inhibitory activity. The plasma of other patients with severe hepatic disease has been

observed to inhibit the activity of thrombin and to retard the clotting of normal blood (18). It is of note that dog plasma, defibrinated by intravenous infusion of thromboplastin, also acquired thrombin-inhibitory properties (2).

SUMMARY

A patient with acquired afibrinogenemia is described. This patient developed acute yellow atrophy of the liver and a fatal bacteremia of *E. coli* var. *communior* in association with an abortion. In addition to complete absence of fibrinogen from the blood, the patient had thrombocytopenia, and her plasma had thrombin-inhibitory properties. Cultures of the organism obtained from the patient's blood were capable of destroying fibrinogen and fibrin *in vitro*. However, fatal experimental infection with this organism did not produce afibrinogenemia in rabbits. The patient's blood did not display abnormal fibrinolytic activity. It seemed most likely that the patient's afibrinogenemia was the result of failure of production of fibrinogen by the severely damaged liver.

BIBLIOGRAPHY

1. Zinsser, H. H. and Williams, W. J. Fibrinolysis in Gram-negative bacilli. J. Bacteriology **58,** 501 (1949)
2. Ratnoff, O. D. and Conley, C. L. Studies on afibrinogenemia II. The defibrinating effect on dog blood of intravenous injection of thromboplastic material. Bull. Johns Hopkins Hosp. **88,** 414, 1951.
3. Morawitz, P. Über einige postmortale Blutveranderungen. Beitr. zur Chem. Physiol. u. Path. **8,** 1 (1906).
4. Goodpasture, E. W. Fibrinolysis in chronic hepatic insufficiency. Bull. Johns Hopkins Hosp. **25,** 330 (1914).
5. Favre-Gilly, J. Les états hémorrhagiques et la notion de fibrinopénie. Lyon, Librairie Medicale, 1947.
6. Henderson, J. L., Donaldson, G. M. M. and Scarborough, H. Congenital afibrinogenemia: report of a case with review of literature. Quart. J. Med. (New Series) **14,** 101 (1945).
7. Corbertt, W. H. A case of congenital afibrinogenemia. Med. J. Australia **1,** 114 (1947).
8. Diamond, L. K. and Borges, W. Congenital afibrinogenemia. Paper presented at International Society of Hematology. Buffalo, N. Y. August 1948.
9. Pinniger, J. L. and Prunty, F. T. G. Some observations on the blood-clotting mechanism. The role of fibrinogen and platelets with reference to a case of congenital afibrinogenemia. Brit. J. Exp. Path. **27,** 200 (1946).

10. WHIPPLE, G. H. Fibrinogen I. An investigation concerning its origin and destruction in the body. Amer. J. Physiol. **33,** 50 (1914).
11. RISAK, E. Die Fibrinopenie. Zeit. fur Klin. Med. **128,** 605 (1935).
12. OPITZ, H. AND SILBERBERG, M. Afibrinogenämie und Thrombopenie infolge ausgedehnter Hepato-lienaler Tuberkulose. Klin. Wchn. **3,** 1443 (1924).
13. WHIPPLE, G. H. AND HURWITZ, S. H. Fibrinogen of the blood as influenced by liver necrosis of chloroform poisoning. J. Exp. Med. **13,** 136 (1911).
14. DRURY, D. R. AND MCMASTER, P. D. The liver as a source of fibrinogen. J. Exp. Med. **50,** 569 (1929).
15. MARDER, M., WEINER, M., SHULMAN, P. AND SHAPIRO, S. Afibrinogenemia occurring in a case of malignancy of the prostate with bone metastases. N. Y. State J. of Med. **49,** 1197 (1949).
16. MOLONEY, W. C., EGAN, W. J. AND GORMAN, A. J. Acquired afibrinogenemia in pregnancy. New Engl. J. Med. **240,** 596 (1949).
17. WEINER, A. E., REID, D. E. AND ROBEY, C. C. Coagulation defects associated with premature separation of the normally implanted placenta. Am. J. Obst. and Gynecol. **60,** 379, 1950.
18. CONLEY, C. L., HARTMANN, R. C. AND MORSE, W. I. II. Circulating anticoagulants: A technique for their detection and clinical studies. Bull. Johns Hopkins Hosp. **84,** 255, 1949.

STUDIES ON AFIBRINOGENEMIA

II. The Defibrinating Effect on Dog Blood of Intravenous Injection of Thromboplastic Material

OSCAR D. RATNOFF and C. LOCKARD CONLEY

Department of Medicine, The Johns Hopkins University and Hospital

Received for publication December 11, 1950

Afibrinogenemia has been observed in a patient following an infected abortion (1). The patient was found to have a bacteremia due to *E. coli* and acute yellow atrophy of the liver. A remarkable feature of this case was the presence of a relatively normal amount of prothrombin in the blood at a time when fibrinogen was absent. It seemed conceivable that infected and necrotic material from the pregnant uterus might have entered the blood and caused intravascular defibrination. For this reason, the effect of intravenous infusion of thromboplastic material was studied in dogs.

In the experiments to be described, it was demonstrated that the intravenous injection of homologous thromboplastin resulted in prolongation of the coagulation time of dog blood. In three of five dogs, the blood became incoagulable because of the absence of fibrinogen. At the same time, the blood acquired thrombin-inhibitory activity. However, the amount of thromboplastin needed to produce these effects was so great as to make it unlikely that clinical afibrinogenemia could occur on this basis.

METHODS AND MATERIALS

Thromboplastin was prepared from dog brain by the technique described by Brambel (2). The resulting suspension was centrifuged at 2500 RPM (rim diameter 13 cm.) for 10 minutes to remove particulate matter. The slightly turbid supernatant fluid was filtered through glass wool into the infusion apparatus.

A mixture of 0.1 ml. of this thromboplastic solution, 0.1 ml. of oxalated normal

This investigation was in part carried out under contract with the Office of Naval Research, United States Navy, and was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

dog plasma and 0.1 ml. of 0.025M calcium chloride solution clotted in 10 to 13 seconds in repeated determinations using different dogs' plasmas.

The *clotting time* of 0.5 ml. portions of whole blood was determined using dry acid-washed pyrex Wassermann tubes at 37°C. Two tubes were used for each determination, and the interval until the formation of a solid clot was recorded as the clotting time.

The concentration of *fibrinogen* in plasma was measured by a method requiring 0.5 ml. of plasma or less. Briefly, a volume of 0.5 ml. of plasma was pipetted into a 50 ml. pyrex centrifuge tube containing 10 ml. of a 0.85 per cent solution of sodium chloride, 0.05 ml. of a solution of thrombin containing 1000 National Institutes of Health units per ml.[1] and approximately 0.5 ml. of crushed pyrex glass. The mixture was agitated during clotting. The fibrin strands adhered to the crushed glass and were separated from the supernatant serum by centrifugation. The fibrin was washed twice with sodium chloride solution, and the amount of fibrin adherent to the glass was then determined by measuring its tyrosine-like activity. The details of this method have been reported separately (3). With this technique, the concentration of fibrinogen in the plasma of the 5 dogs studied varied from 153 to 630 mg. per 100 ml. before the performance of any procedure except anesthetization. This wide variation in the basal level of fibrinogen in dog plasma was noted by Whipple (4) and others.

The *prothrombin time* was the time which lapsed until clotting when 0.1 ml. of oxalated dog plasma was mixed with 0.1 ml. of rabbit brain thromboplastin and 0.1 ml. of 0.025 M calcium chloride solution. The plasma was obtained from blood which was oxalated by mixing 9 volumes of blood with 1 volume of 0.1 M sodium oxalate. The prothrombin time of undiluted plasma and of plasma diluted with four volumes of 0.85 per cent sodium chloride solution were determined. Since the prothrombin time may be influenced by extremely low levels of fibrinogen in the specimen tested, the prothrombin time was also determined on certain specimens by diluting plasma with four volumes of normal dog plasma, from which prothrombin had been adsorbed with barium sulfate.

The *thrombin time* was determined by the addition of 0.1 ml. of a solution of thrombin, containing 8 National Institutes of Health units per ml., to 0.1 ml. of canine plasma, and recording the interval until the formation of a clot.

Thrombin-inhibitory activity was measured by the addition of 0.1 ml. of a solution of thrombin, containing 8 units per ml., to 0.1 ml. of oxalated normal dog plasma mixed with 0.1 ml. of the oxalated plasma to be tested. The interval until the formation of a clot was recorded and compared with the clotting time of a mixture of 0.1 ml. of normal dog plasma, 0.1 ml. of 0.85 per cent sodium chloride solution, and 0.1 ml. of thrombin solution.

The *sedimentation rate* was determined by the method of Wintrobe and Landsberg (5).

[1] Thrombin was obtained through the courtesy of Parke Davis Co., Detroit.

EXPERIMENTAL DATA

A mongrel dog weighing 11.5 kg. was anesthetized by the intravenous administration of 0.3 gm. of sodium pentobarbital (Nembutal, Abbott) and anesthesia was maintained for 6½ hours by repeated injections of 30 to 60 mg. of sodium pentobarbital. A preparation of homologous thromboplastin was infused from a burette through a 20 gauge needle inserted into the left saphenous vein. One tenth ml. of this dog's oxa-

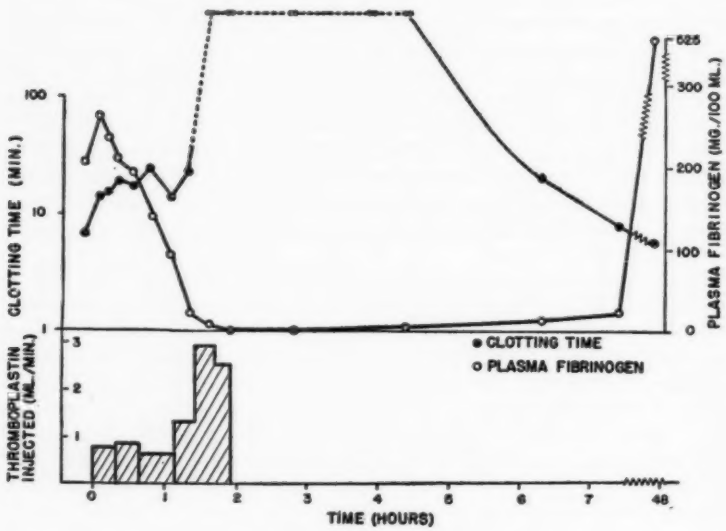


FIG. 1

lated plasma was clotted in 10 and 11 seconds (duplicate determinations) on the addition of 0.1 ml. of the thromboplastic preparation and 0.1 ml. of 0.025M calcium chloride solution.

One hundred forty-nine ml. of this thromboplastic preparation were infused at a variable rate over a period of 115 minutes (Fig. 1). The rate of injection averaged 0.72 ml. per minute during the first 69 minutes, and 2.15 ml. per minute during the remaining 46 minutes. Using dry needles and syringes, blood was drawn from an external jugular vein at intervals before, during and after infusion. The clotting

time, fibrinogen content, prothrombin time, thrombin time, thrombin-inhibitory activity, icterus index, sedimentation rate, and hematocrit were determined. During the time of the infusion, the dog's general condition appeared unaltered, and there were no convulsions or involuntary movements. Eighty-four minutes after the onset of the infusion, hematomas were first observed to develop at the sites of venepunctures. There was no other evidence of abnormal bleeding. No evidence of thrombosis was observed, either in the vein into which the thromboplastic material was injected or in other superficial veins. On the day following the infusion, the dog appeared weak, but no other abnormalities were noted. Thereafter, the animal appeared normal in every respect during a period of observation of more than two weeks.

The results of this experiment are shown in Fig. 1 and in Tables I–IV. Ninety-six minutes after the beginning of the infusion, the blood was incoagulable because of the absence of fibrinogen. Blood drawn 2½ hours after the infusion was discontinued clotted poorly and after a considerable delay. Three hours later, the clotting time had returned to normal, but the amount of fibrinogen found was small. Two days later the clotting time was again normal, and the fibrinogen concentration was elevated above the pre-injection level (Table I).

The rate at which the dog's oxalated plasma was clotted by bovine thrombin was measured. During the infusion, there was a progressive prolongation of the thrombin clotting time until the plasma became incoagulable (Table II). The prolongation of the thrombin clotting time was not entirely attributable to the decrease in plasma fibrinogen. The rate of clotting of normal oxalated dog plasma by thrombin was retarded by the plasma of the dog under study (Table III). This thrombin-inhibitory activity decreased after the infusion of thromboplastin was terminated. Because of the presence of thrombin-inhibitory activity in the dog's plasma, it was not possible to estimate reliably the prothrombin concentration of the plasma. However, the data suggest that there was no appreciable reduction of the plasma prothrombin concentration (Table IV). For the same reason, the concentration of accelerator globulin could not be measured accurately, but no evidence was obtained that its concentration was reduced.

In summary, the blood of a dog was made transiently incoagulable by the intravenous infusion of homologous thromboplastin. The clot-

ting defect was due to the disappearance of fibrinogen from the plasma. At the same time, the plasma acquired mild thrombin-inhibitory activity. These alterations occurred without external evidence of intravascular clotting, and the dog survived without apparent difficulty.

TABLE I

Effect of Infusion of Thromboplastin on the Rate of Clotting and Concentration in Plasma of Fibrinogen

TIME	CLOTTING TIME IN DUPLICATE	CONCENTRATION OF FIBRINOGEN	SED. RATE	HEMATOCRIT	ICTERUS INDEX
	min.	*mg/100 ml.*	*mm./1 hr.**	*per cent*	*Meulengracht units*
Pre-infusion	3, 6½	207	0	49.0	5
0 Thromboplastin infusion begun	—	—	—	—	—
5 min.	8½, 14½	263	1.0	49.0	5
12½ min.	11, 15½	238	1.0	49.0	4
20½ min.	13, 18½	212	2.0	50.0	5
34½ min.	12½, 17½	191	1.0	53.5	5
46½ min.	22, 24½	139	1.0	50.5	5
1 hr. 5 min.	11½, 14	92	1.0	51.0	6
1 hr. 21 min.	12, 24†	27	0.5	52.0	Hemolyzed
1 hr. 36½ min.	∞, ∞	0‡	0.2	50.0	7.5
1 hr. 55 min. Thromboplastin infusion completed	—	—	—	—	—
1 hr. 57 min.	∞, ∞	0‡	0.1	49.5	8
2 hr. 49 min.	∞, ∞	0‡	0.1	54.0	7
3 hr. 57½ min.	∞, ∞	—	—	—	—
4 hr. 25 min.	∞, ∞	0‡	0	54.0	Hemolyzed
6 hr. 20 min.	6,† 20†	14	0.2	55.0	Hemolyzed
7 hr. 25 min.	6½,† 8†	25	—	55.0	5
48 hr.	6, 6	525	10.0	42.0	4

* Uncorrected.
† Partial clots.
‡ Too low to read.

The experiment was repeated on four other dogs, using different rates of administration of thromboplastin. The survival of the animals appeared to depend upon the rate at which the thromboplastic material was infused during about the first sixty minutes. Thus, 2 dogs, infused at the rate of 1.22 and 1.85 ml. per minute died 43 and 18 minutes

respectively after the start of the infusion. One of these dogs died before defibrination was complete. At autopsy, these two dogs were found to have large clots occluding the tricuspid and pulmonary orifices of the heart. No clots were seen in the left side of the heart. In a third animal, relatively small amounts of thromboplastin were infused, 0.23 ml. per minute for a period of 95 minutes. The fibrinogen concen-

TABLE II

Effect of Infusion of Thromboplastin on the Rate of Clotting of Oxalated Dog Plasma by Thrombin

TIME	CLOTTING TIME*
	seconds
Pre-infusion	15, 14
0 Thromboplastin infusion begun	—
5 min.	15, 16
12½ min.	17, 16
20½ min.	16, 16
34½ min.	19, 20
46½ min.	23, 21
1 hr. 5 min.	31, 32
1 hr. 21 min.	64,† 65†
1 hr. 36½ min.	∞, ∞
1 hr. 55 min. Thromboplastin infusion completed	—
1 hr. 57 min.	∞, ∞
2 hr. 49 min.	∞, ∞
4 hr. 25 min.	∞, ∞
6 hr. 20 min.	60,† 60†
7 hr. 25 min.	25,† 27†
48 hr.	13, 13

* 0.1 ml. oxalated dog plasma plus 0.1 ml. solution of Thrombin containing 5 N. I. H units per ml. Duplicate determinations.

† Partial clots.

tration in the plasma of this dog at no time decreased below 378 mg. per 100 ml. from an initial level of 630 mg. per 100 ml., although the clotting time was greatly prolonged. This dog died 3 days after the infusion. A fourth dog received the thromboplastin for 134 minutes at an average rate of 0.91 ml. per minute. His blood became incoagulable after 90 minutes, at which time fibrinogen was not detectable in the plasma. The dog died on the day following the infusion. An autopsy showed non-obstructive clots in both ventricles along the chordae ten-

dinae and trabeculae, but it was not established whether these had occurred *postmortem*.

In two dogs, then, defibrination occurred without immediately fatal results. It is of interest that the rate of infusion was 0.72 and 0.58 ml. per minute during the first 69 and 47 minutes respectively in these two animals. After defibrination had been achieved it was possible to

TABLE III

Effect of Plasma of a Dog Infused with Thromboplastin on the Rate of Clotting of Normal Dog Plasma by Thrombin

TEST PLASMA	CLOTTING TIME*
	seconds
Control: 0.1 ml. 0.85 per cent sodium chloride solution	20, 22
Pre-infusion	18, 19
0 Thromboplastin infusion begun	—
5 min.	18, 19
34½ min.	18, 21
46½ min.	22, 23
1 hr. 5 min.	30, 31
1 hr. 21 min.	32, 36
1 hr. 36½ min.	30, 30
1 hr. 55 min. Thromboplastin infusion completed	—
1 hr. 57 min.	26, 30
2 hr. 49 min.	26, 28
4 hr. 25 min.	26, 28
6 hr. 20 min.	25, 25
7 hr. 25 min.	24, 26

* 0.1 ml. Solution of thrombin containing 5 N. I. H. units added to mixture of 0.1 ml. normal dog plasma and 0.1 ml. plasma of dog infused with thromboplastin. Duplicate determinations.

infuse these two dogs with thromboplastic material at rates which presumably would have been lethal had they been used from the start. After defibrination, the rate of injection was 2.50 ml. and 1.29 ml. per minute.

In each dog, there was a striking prolongation of the clotting time of whole blood during the infusion. In no instance was this preceded by a period during which the clotting time was observed to be appreciably shortened. The fibrinogen concentration of the plasma was reduced in every animal, and in 3 of the 5, complete absence of fibrinogen

TABLE IV

Effect of Infusion of Thromboplastin on the Prothrombin Time of Dog Plasma

TIME		CLOTTING TIME OF UNDILUTED PLASMA*	CLOTTING TIME OF PLASMA DILUTED WITH 4 VOLUMES OF SALINE SOLUTION†	CLOTTING TIME OF PLASMA DILUTED WITH 4 VOLUMES OF PROTHROMBIN-FREE NORMAL DOG PLASMA‡
		seconds	*seconds*	*seconds*
Pre-infusion		8, 9	14, 15	12, 13
0	Thromboplastin infusion begun	—	—	—
5 min.		8, 9	14, 17	—
12½ min.		8, 9	18, 18	—
20½ min.		9, 9	17, 19	—
34½ min.		9, 10	20, 23	—
46½ min.		10, 10	23, 23	—
1 hr. 5 min.		11, 12	28, 32	—
1 hr. 21 min.		11, 13	56,§ 54§	13, 15
1 hr. 36½ min.		∞, ∞	∞, ∞	—
1 hr. 55 min.	Thromboplastin infusion completed	—	—	—
1 hr. 57 min.		∞, ∞	∞, ∞	17, 17
2 hr. 49 min.		∞, ∞	∞, ∞	—
4 hr. 25 min.		∞, ∞	∞, ∞	—
6 hr. 20 min.		20,§ 21§	95,¶ 100¶	—
7 hr. 25 min.		15,§ 17§	80,¶ 80¶	—
48 hr. 0 min.		8, 8	13, 13	—

* 0.1 ml. 0.025M $CaCl_2$ solution added to a mixture of 0.1 ml. oxalated test plasma and 0.1 ml. rabbit brain thromboplastin. Duplicate determinations.

† 0.1 ml. 0.025M $CaCl_2$ solution added to a mixture of 0.1 ml. oxalated test plasma (diluted with 4 volumes of 0.85 per cent sodium chloride solution) and 0.1 ml. rabbit brain thromboplastin. Duplicate determinations.

‡ 0.1 ml. 0.025M $CaCl_2$ solution added to a mixture of 0.1 oxalated test plasma (diluted with 4 volumes of $BaSO_4$ adsorbed normal dog plasma) and 0.1 ml. rabbit brain thromboplastin. The $BaSO_4$ adsorbed plasma was prepared by incubating oxalated normal dog plasma at 37°C. for 10 minutes with one-tenth its volume of powdered barium sulfate. After centrifugation, the supernatant plasma failed to clot upon the addition of thromboplastin and calcium. Duplicate determinations.

§ Partial clots.

¶ A few fibrin strands.

was observed. In the four animals on whose blood frequent measurements were made, it was apparent that marked prolongation of the clotting time preceded a significant fall in fibrinogen. Moreover, in each of the 4 animals tested, thrombin-inhibitory activity appeared

in the plasma, but only after an appreciable fall in the concentration of plasma fibrinogen had occurred. It was therefore not possible to account for the early prolongation of the clotting time in these animals.

The experiments described demonstrated that the intravenous injection of homologous thromboplastic material into dogs prolonged the clotting time of shed blood, and in 3 of 5 animals, rendered the blood incoagulable. Similar observations were made by Woolridge (6), Mills (7), Copley (8), Thomas (9), Schneider (10) and Fulton and Page (11) who injected thromboplastic tissue extracts intravenously into dogs (6, 7, 8), rabbits (6, 7, 9), cats (6) and mice (9, 10, 11). It was possible to show that prolongation of the clotting time was associated with a decrease in the amount of circulating fibrinogen, and no fibrinogen was detected in blood which failed to clot. Presumably, the fibrinogen of the plasma was converted *in vivo* into fibrin. This hypothesis seems likely, since the dogs which were infused with thromboplastin at the greatest rate died quickly, and at autopsy, large intravascular clots were demonstrable. Moreover, other clot promoting agents, when injected intravenously, have been reported to make the blood incoagulable. Thus, blood drawn after the injection of thrombin (12), viper venom (13), or trypsin (14) failed to clot.

There is indirect clinical evidence that intravascular clotting is a continual occurrence. Foster and Whipple (15) pointed out many years ago that when defective coagulation occurs, bleeding about the teeth, in the gastro-intestinal tract, and the subcutaneous tissues is common. This suggests that small injuries are constantly present in these areas, and are normally repaired by fibrin clots. Thus, fibrinogen may be constantly utilized by its conversion to fibrin.

The question arises whether afibrinogenemia may result from excessive deposition of fibrin. Extensive injuries are not associated with decrease in circulating fibrinogen, nor is there any evidence that clinical afibrinogenemia occurs as the result of introduction of thromboplastic material into the blood stream. Afibrinogenemia has been reported in association with disorders of pregnancy, (16, 17). Since the placenta is a rich source of thromboplastic substance (8), it is possible that these rare cases are the result of intravascular defibrination. However, no data are available to support this thesis. It is of note that the amount of thromboplastic material necessary to achieve defibrination in dogs

is relatively large. A dog's total volume of blood could be clotted *in vitro* in less than one minute by the amount of thromboplastin required to effect defibrination *in vivo*.

In earlier descriptions of the results of intravenous injections of thromboplastic substances, considerable emphasis was placed on the observation that small injections of thromboplastin accelerated clotting. With the preparation of thromboplastin used, no shortening of the clotting time was observed in the present study. The explanation of this difference in result is not clear. In each of 4 animals studied, the plasma acquired thrombin-inhibitory properties when the concentration of fibrinogen was decreased. Possibly the thromboplastic material itself inhibited the action of thrombin. It has been demonstrated *in vitro* that brain thromboplastin may have thrombin-inhibitory properties (18). It is of interest that the plasma of the patient with acquired afibrinogenemia associated with hepatic damage, described in the preceding paper, also inhibited the action of thrombin (1).

SUMMARY

An intravenous infusion of homologous thromboplastic material was administered to dogs. When the thromboplastin was injected at a rapid rate, the dogs died, and at autopsy the mitral and pulmonary orifices were occluded with thrombi. When the thromboplastin was infused more slowly, a transient, non-fatal afibrinogenemia could be induced. Moreover, in dogs infused with thromboplastic material, the clotting time was often prolonged out of proportion to the decrease in concentration of fibrinogen; and the plasma acquired thrombin inhibitory properties.

BIBLIOGRAPHY

1. Conley, C. L., Ratnoff, O. D., Hartmann, R. C. Studies on Afibrinogenemia I. Afibrinogenemia in a patient with septic abortion, acute yellow atrophy of the liver and bacteremia due to E. coli. Bull. Johns Hopkins Hosp. **88,** 402 (1951).
2. Brambel, C. E. Thromboplastic reagent. Development of a more suitable preparation for measuring accelerated clotting tendency and for use following administration of dicoumarin. Arch. Surg. **50,** 137 (1945).
3. Ratnoff, O. D. and Menzie, C. A new method for the determination of fibrinogen in small samples of plasma. To be published.

4. WHIPPLE, G. H. Fibrinogen I. An investigation concerning its origin and destruction in the body. Amer. J. Physiol. **33,** 50 (1914).
5. WINTROBE, M. M. AND LANDSBERG, J. W. Standardized technique for blood sedimentation test. Amer. J. Med. Sci. **189,** 102 (1935).
6. WOOLRIDGE, L. C. Über intravasculare Gerinnungen. Arch. Anat. u. Physiol. (Physiol. Abt.) p. 397 (1886).
7. MILLS, C. A. The action of tissue extracts in the coagulation of blood. J. Biol. Chem. **46,** 167 (1921).
8. COPLEY, A. L. Studies on human placental thromboplastin *in vitro* and *in vivo*. Science **101,** 436 (1945).
9. THOMAS, L. Studies on the intravascular thromboplastic effect of tissue suspensions in mice. I. The reaction of mice to intravenous injections of a sedimentable tissue component. Bull. Johns Hopkins Hosp. **81,** 1 (1947).
10. SCHNEIDER, C. L. The active principle of placental toxin: thromboplastin; its inactivation in blood: antithromboplastin. Amer. J. Physiol. **149,** 123 (1947).
11. FULTON, L. D. AND PAGE, E. W. Nature of the refractory state following sublethal doses of human placental thromboplastin. Proc. Soc. Exp. Biol. and Med. **68,** 594 (1948).
12. JÜRGENS, R. AND STUDER, A. Zur Wirkung des Thrombins. Helv. Physiol. et Pharmacol. Acta **6,** 134 (1948).
13. MELLANBY, J. The coagulation of blood, Part II. The actions of snake venoms, peptone and leech extract. J. Physiol. **38,** 441 (1909).
14. JOBLING, J. W., PETERSEN, W. AND EGGSTEIN, A. A. Snake ferments and antiferments during trypsin shock. J. Exp. Med. **22,** 141 (1915).
15. FOSTER, D. P. AND WHIPPLE, G. H. Blood fibrin studies III. Fibrin values influenced by transfusion, hemorrhage, plasma depletion and blood pressure changes. Amer. J. Physiol. **58,** 393 (1921).
16. MOLONEY, W. C., EGAN, W. J. AND GORMAN, A. J. Acquired afibrinogenemia in pregnancy. New Engl. J. Med. **240,** 596 (1949).
17. WEINER, A. E., REID, D. E. AND ROBEY, C. C. Coagulation defects associated with premature separation of the normally implanted placenta. Am. J. Obst. and Gynecol. **60,** 379 (1950).
18. HARTMANN, R. C. The thrombin-inhibitory activity of certain thromboplastin preparations. To be published.

STREPTOMYCIN IN THE TREATMENT OF TUBERCULOUS PERICARDITIS

DOUGLAS CARROLL

Department of Medicine, The Johns Hopkins University School of Medicine, and The Chest Service, Columbia University Division of Bellevue Hospital, New York City

Received for publication December 15, 1950

Several reports of the effect of streptomycin in tuberculous pericarditis have appeared recently (1, 2, 3, 4), but clearcut indications for its use must await increased experience. Information concerning the course of untreated acute tuberculous pericarditis with which to compare the course of streptomycin-treated cases is lacking. It is the purpose of this paper to analyse the manifestations of tuberculous pericarditis in 71 untreated cases, previously described (5) and to report a small number of patients treated with streptomycin.

1. *Pathogenesis and Course of Acute Tuberculous Pericarditis:* A study of the records of 110 cases of tuberculous pericarditis supports the suggestion (5, 6, 7, 8, 9) that the pericardium is usually infected from the mediastinal lymph nodes rather than from the blood stream (10).

Following the introduction of tubercle bacilli into the pericardium, there may be a stage of pericarditis without fluid formation, but if the lesion is not localized, a pericardial effusion results. The effusion is gradually absorbed, leaving a fibrous sac surrounding the heart. Symptoms of cardiac compression may not be present in any of these stages, and several of our cases passed through the acute stage of tuberculous pericarditis with only mild systemic complaints.

The factors of outstanding importance in the prognosis of tuberculous pericarditis are first, the extent of tuberculosis elsewhere in the body, and second, the degree of cardiac constriction.

2. *Follow-up Study on 71 Patients with Clinically Significant Tuberculous Pericarditis not Treated with Streptomycin:* In 1937 Harvey and Whitehill (5) reported 71 cases of symptomatic tuberculous pericarditis seen at The Johns Hopkins Hospital. Recently an attempt has been made to determine the fate of each of these patients.

A. Early Deaths: Of this group, 39 patients died within 2 years of the onset of the pericarditis. Thirty-three of these patients had widespread tuberculosis either clinically or at autopsy. Cardiac failure was present terminally in 20, and in 9 was severe. In only 6 of the patients who died was the tuberculosis mainly localized in the pericardium and mediastinum. In 4 of these 6, no pericardiocentesis had been done; they showed respectively 1,000 cc., 750 cc., 425 cc. and 500 cc. of pericardial fluid at autopsy. In two patients in whom pericardiocenteses had been performed, there were 3,500 cc. and 1,500 cc. of pericardial fluid at autopsy. It is concluded that widespread tuberculosis is the main cause of death in tuberculous pericarditis and that cardiac tamponade is fatal less often.

B. Five Year Survivals: Eighteen patients who were discharged from the hospital could not be traced, but 14 patients survived for 5 years or longer. Thus from a total group of 71 patients with symptomatic tuberculous pericarditis, at least 19 per cent survived for 5 years and were well.

The follow-up study on the patients from the series of Harvey and Whitehill provided an opportunity to estimate the survival rate of tuberculous pericarditis after 5 years. The additional question arose of how many survivors would develop pericardial constriction in later years. It was possible to find 7 more patients who had survived tuberculous pericarditis for 5 or more years. (These 7 patients could not be included in the analysis of percentage survival since the size of the group from which they were drawn was unknown.)

The data on the 21 cases (14 from the series of Harvey and Whitehill,* and 7 from other sources†) are presented in Charts 1 and 2. These patients were followed for an average period of 12.6 years after the onset of tuberculous pericarditis. Chart 2 summarizes the findings at the time of follow-up. Three patients had abnormal electrocardiograms, one had been operated on for chronic constrictive pericarditis, and one had calcification of the pericardium without cardiac symptoms. Five patients died after the five year period of follow-up, two of tuberculosis in the seventh year after the onset of pericarditis, two of carcinoma of the prostate and one of carcinoma of the stomach.

*The cases from Harvey and Whitehill's series are included in parentheses in the Charts.

† The 7 additional cases are not in parentheses in the Charts.

CHART 1

CHART 1

Summary of Findings on Nineteen Patients in Whom a Diagnosis of Acute Tuberculous Pericarditis was Made and Who Survived for a Period of Five Years

Data applies to the first admission, except that tuberculosis discovered at any time was included in lowest horizontal line.

	1	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	13	14	15	(16)	(17)	18	(19)	20	21
Age	16	46	13	22	20	42	18	45	13	28	42	20	34	19	19	54	14	28	60	37	29
Sex	M	M	F	M	F	M	M	F	F	M	M	M	F	M	F	M	M	F	M	F	F
Race	W	C	C	C	C	W	C	C	C	C	C	C	C	C	C	C	C	W	C	C	C
Cardiac symptoms	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+
Tuberculosis symptoms	+	0	+	+	+	0	+	+	+	+	0	+	0	+	+	+	+	+	0	+	0
Weight loss*	+	0	0	0	20	18	22	+	+	0	0	0	0	+	0	0	10	+	+	25	0
Pulsus paradoxus	+	0	0	0	+	0	0	0	+	0	0	+	+	0	0	0	+	0	+	+	+
Pericardial friction rub	+	+	+	+	+	+	+	+	+	+	+	+	0	0	+	+	0	+	0	+	+
Distant heart sounds	0	+	+	0	0	0	0	0	+	0	0	+	+	+	0	+	+	+	+	+	+
Liver enlargement	0	0	0	0	0	+	0	0	+	0	0	+	+	0	0	0	+	+	+	0	0
Initial leucocyte count†	8.4	6.0	4.8	8.1	12.0	12.6	11.6	5.8	8.5	7.5	5.4	8.1	4.8	–	6.0	5.7	6.0	6.6	4.4	5.8	9.4
Chest x-ray suggestive of pericardial effusion	+	–	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	0	+	+	0
Electrocardiogram compatible with pericarditis	–	–	+	0	+	0	0	+	+	+	+	+	+	+	+	+	+	+	0	+	+
Days temperature over 100°	18	0	44	0	70	4	30	49	38	21	62	31	59	–	32	29	24	90	0	15	44
Pericardial tap	0	0	0	0	0	0	2	0	1	1	0	1	1	0	0	0	1	0	2	1	0
Mediastinal tuberculosis by x-ray	0	0	0	0	+	0	+	0	0	+	0	0	0	0	+	+	+	0	+	0	+
Minimal pulmonary tuberculosis by x-ray	0	0	0	0	+	0	0	+	0	0	+	0	0	0	+	0	+	0	0	0	0
Moderately advanced pulmonary tuberculosis by x-ray	0	0	0	0	0	0	0	0	+	0	0	0	0	+	0	0	0	+	0	0	0
Tuberculosis peritonitis	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0
Pleurisy with effusion	+	0	0	+	0	0	0	+	0	0	0	0	0	0	0	+	0	+	0	+	0
Tuberculosis elsewhere	0	0	0	0	0	0	0	0	0	0	0	+	0	+	0	+	0	+	0	0	0

+ Finding present.
0 Finding not present.
– Procedure not done or unknown.
* Weight loss in pounds where number is present.
† Leucocytes in thousands.
( ) Case originally reported by Harvey and Whitehill.

The history of the patient who developed constrictive pericarditis is of sufficient interest to present in more detail:

H. T., a 16 year old white boy was admitted to The Johns Hopkins Hospital in May of 1914 complaining of weakness of 5 months' duration. He also had a heavy feeling in the chest associated with mild substernal discomfort.

On examination, bilateral enlargement of the cervical and axillary glands was present. The lungs were clear. There was a localized impulse in the fifth inter-

CHART 2

Follow-Up Data on Twenty-one Patients with a Clinical Diagnosis of Acute Tuberculous Pericarditis

Patient No. 1 developed constrictive pericarditis with calcification of the pericardium.
Patient No. 14 developed calcification of heart without signs of cardiac failure.
Patient No. 19 had amyloid deposits in the heart at autopsy.

Case	1	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	13	14	15	(16)	(17)	18	(19)	20	21
Length of follow-up in years	26	26	21	17	16	15	15	14	13	12	12	12	10	9	8	7	7	7	6	6	5
Clinical state	F	D	N	N	H	N	N	D	N	N	H	N	H	T	N	D	D	T	D	N	N
Chest plate	E	–	N	T	N	–	N	–	T	N	N	N	H	T	N	T	T	T	N	N	–
Cause of death		C						C								T	T		C		
ECG	F	–	N	–	N	N	N	–	–	N	N	•	N	•	N	–	N	–	–	–	–

F Fibrillating (Case 1).
D Dead.
N Normal.
H Hypertension.
T Tuberculosis.
C Cancer.
– Procedure not done.
• T1 isoelectric, T2 inverted.
E Enlarged heart.

space just outside the left nipple. The area of cardiac dullness extended 11 cm. to the left of the midsternal line in the fifth interspace. The heart sounds were distant. There was a systolic pericardial friction rub to the right of the lower sternum. The liver and spleen were not felt. There was no ankle edema or ascites. A paradoxical pulse was present. The white blood count was 8,900.

There was a low-grade fever for three weeks, with a sinus tachycardia of about 120. X-ray of the chest revealed cardiac enlargement with prominent mediastinal glands. A pleural effusion developed on the right and thoracentesis was carried out twice. The signs and symptoms disappeared in one month. A diagnosis of tuberculous pericarditis was made.

The cervical glands gradually enlarged, and, in 1917, a biopsy showed tuberculosis.

The patient was well until 1935 when he developed dyspnea, cough and weak-

ness. Fluid removed from the right pleural cavity at this time contained tubercle bacilli.

In March of 1936, study in another hospital showed enlargement of the radiographic shadow of the heart with areas of calcification in the pericardium. There was fluid in the right pleural space. The liver was felt 10 cm. below the right costal margin. Right axis deviation and auricular fibrillation were demonstrated by the electrocardiogram. Ankle edema and exertional dyspnea developed while in the hospital.

On July 25th, a pericardectomy was performed. The pericardium was thick and adherent with a hard calcified plaque attached. Examination of the material removed at operation showed scar tissue only. Following operation there were no more symptoms of cardiac failure.

In April of 1945 he entered The Johns Hopkins Hospital with a typical history of intermittent biliary colic during the preceding five months. The heart was enlarged to the left anterior axillary line. A mass was felt in the right upper quadrant of the abdomen extending 3 cm. below the costal margin. The heart was symmetrically enlarged by X-ray, and calcification of the lymph nodes of the left side of the neck was present. Gall stones were visualized on the flat plate of the abdomen. The circulation time was 60 seconds, and the venous pressure 234 mm. of saline above the sternum. The electrocardiogram showed auricular fibrillation and marked right axis deviation.

On April 20, 1945, a cholecystectomy was performed. The liver showed gross evidence of cirrhosis. A liver biopsy revealed scarring of the liver, continuous with the liver capsule.

Three months later the patient had no signs or symptoms of cardiac failure.

Comment: This patient went through the complete course of tuberculous pericarditis from the acute effusive stage to that of calcification of the pericardium with constrictive pericarditis 22 years later. That this is an unusual course for acute tuberculous pericarditis is suggested by the rarity of such cases in the literature.

3. *Use of Streptomycin in Tuberculous Pericarditis:* It is apparent from the foregoing analysis that control of widespread tuberculosis and relief of pericardial constriction are the most important measures in the treatment of tuberculous pericarditis. Streptomycin is the most effective drug in controlling widespread tuberculosis, whereas surgical procedures (pericardiocentesis for acute cardiac tamponade, and pericardectomy for constrictive pericarditis) are the definitive methods of relieving the severe cardiac symptoms of tuberculous pericarditis. In patients with widespread tuberculosis and severe constrictive peri-

carditis, the use of streptomycin may allow earlier surgical intervention because of improved operative condition. The following case reports illustrate some of the uses of streptomycin and problems in the treatment of tuberculous pericarditis:

R. C., a 21 year old colored housewife was referred to Bellevue Hospital for her second admission on April 28th, 1948, because of loss of weight and fever of two weeks' duration.

She had been admitted to Bellevue Hospital once previously on August 25, 1947. At that time, there was a history of swelling of the abdomen of three months' duration. The temperature was 102°F., the pulse 120, the respirations 18, and the blood pressure 116/60. The lungs were normal on physical examination. The point of maximal cardiac impulse was in the fifth intercostal space, just outside the midclavicular line. The sounds were of good quality, and no murmurs were heard. There was fullness of the abdomen and shifting dullness in the flanks.

Abdominal paracentesis was done on August 29th with the removal of 1,600 cc. of fluid which was positive on culture for acid fast bacilli. There was fever between 100° and 101° F. Streptomycin 2.0 Gm. daily was started on September 20th. The temperature returned to normal within two days. Streptomycin was continued for 33 days. There was a gain of 33 pounds in weight. She was allowed to return home to be followed in the clinic. There were no signs of symptoms of tuberculosis elsewhere.

In April, 1948, at follow-up clinic she was febrile and was admitted for study. There had been 7 pounds loss of weight in several weeks, but she felt quite well. She denied having fever, nightsweats, or weakness.

On examination the temperature was 99.6° F., the pulse 90, the respirations 18, and the blood pressure 120/64. The area of precordial dullness was not increased, the point of maximal cardiac impulse could be easily seen and felt in the fifth intercostal space. The heart sounds were not distant. There was no murmur or friction rub. The liver was felt one cm. below the right costal margin. There was no ascites, distention of cervical vessels, edema of the ankles or cyanosis.

On admission the venous pressure and circulation time were normal. There was a gradual increase in the size of the heart and a slow development of ascites. The second pulmonic sound became louder than the second aortic sound.

There was a low grade fever until early June when the temperature returned to normal. The X-ray of the chest at that time showed an enlarged heart with small pulsations of the lower cardiac borders on kymography. The electrocardiogram when compared with one taken on the first admission showed low voltage with inversion of T waves in V3 and V5.

On July 27, 1948, dyspnea suddenly appeared, followed by nausea and vomiting. The temperature was 98.8°, the pulse 160, and the respiratory rate 36. There was a paradoxical pulse, and the blood pressure was 116/60. No point of maximal impulse was seen or felt. The area of precordial dullness was increased both to the

left and right. The heart sounds were distant. The liver edge was felt one cm. below the costal margin. The venous pressure was 210 mm. of saline solution.

A pericardiocentesis yielded 250 cc. of sanguinous fluid. One hundred mgm. of streptomycin were injected into the pericardial space. There was dramatic relief of all discomfort. The paradoxical pulse disappeared, and the respiratory rate returned to 18 per minute. The venous pressure dropped to 105 mm. of saline solution. Examination of the pericardial fluid showed an erythrocyte count of 2.46 million per cubic mm. and a leucocyte count of 600 per cubic mm. with 97 percent lymphocytes.

Treatment was begun with one Gm. of streptomycin daily. On the 29th, a second pericardial tap was necessary. Two hundred fifty cc. of fluid were removed and found to contain 6,000 leucocytes, all mononuclears. This pericardial tap again relieved the symptoms completely.

By early August, a marked reduction in the size of the heart was noted by X-ray. The ascites gradually cleared without paracentesis. The venous pressure and circulation time remained normal. The electrocardiogram showed inversion of Tl. ST3 was slightly elevated. The liver edge was not felt after August 12th. At this time the patient felt perfectly well and had been afebrile for more than three months. She felt she was cured and left the hospital against advice.

Comment: This patient presents the classical picture of symptomatic acute tuberculous pericarditis. Treatment with streptomycin was started early in the course of the disease. Unfortunately, cultures of the pericardial fluid were not done. The dramatic symptoms of cardiac tamponade were relieved twice by pericardial tap. A second episode of tamponade occurred two days after streptomycin had been started.

There was widespread tuberculosis as evidenced by tuberculous peritonitis followed by pericarditis. Streptomycin was evidently effective in limiting the further spread of the tuberculosis, but a longer follow-up will be necessary to determine how permanent the therapeutic result was.

R. Br., an 18 year old colored boy, was admitted to Bellevue Hospital January 19, 1948 with a history of 6 months of painless lymphadenopathy in the neck. He also had night sweats and persistent cough.

He had had several previous admissions for disorders of behavior and was sent to the psychiatric ward for observation.

On examination there was a mass of matted rubbery lymph nodes 5 cm. in diameter in the right posterior cervical triangle and a smaller mass in the left posterior cervical chain. There was no distention of the cervical vessels. The cardiac impulse could be seen in the fifth intercostal space at the left midclavicular

line. The sounds were of good quality, and no murmurs were heard. The liver was not palpable. There was no edema, ascites or clubbing of the digits.

Late in February, an elevation of temperature between 100 and 102° F. was noted. The area of cardiac dullness was found to extend into the left axilla. The pulse rose to around 140 per minute. Biopsy of a lymph node from the left posterior cervical chain revealed tuberculosis. Shortly thereafter an X-ray of the chest showed an enlarged bag-shaped heart shadow. On March 3rd, the venous pressure was 250 mm. of saline and the circulation time with decholin was 28 seconds. A diagnosis of acute tuberculous pericarditis was made. The electrocardiogram showed a sinus tachycardia of 140, right axis deviation, T inverted in Leads I, II, V5, and V6. This electrocardiographic pattern did not change significantly throughout his stay in the hospital.

By March 12th, the heart sounds had become distant and tictac in quality. The second pulmonic sound exceeded the second aortic sound in intensity. The blood pressure was 98/72. The liver was felt 3 cm. below the right costal margin, and the spleen was palpable.

In the first two weeks of March the temperature was between 101 and 104°. The administration of one Gm. of streptomycin daily was started on March 15th, followed within several days by a dramatic drop of temperature to between 100 and 101°. There was no change in the size of the heart by X-ray, and no slowing of the heart rate. It was felt that the patient had widespread tuberculosis involving the mediastinum, pericardium, spleen and lymph nodes, and that treatment with streptomycin should be reserved for more serious complications. Streptomycin, therefore, was discontinued after 15 days.

Paradoxical pulse was first noted on May 22nd.

The liver continued to increase in size; fluid began to accumulate in the right side of the chest. This was negative on culture for acid fast bacilli. Because of the increasing signs of pericardial constriction, surgical intervention was considered. Pericardiocentesis failed to yield fluid. A low grade fever was felt to be a contraindication to a pericardectomy. In October the temperature rose to 104° F.; there was an increased cough and pulse rate. A nodular infiltration in the lower lobe of the left lung was discovered in a roentgenograph of the chest. Streptomycin 1 Gm. daily was resumed on October 29th, but subsequently signs of tuberculosis in the sternum, brain and acromial end of the right scapula appeared, and the patient was transferred to a sanitarium for terminal care.

Comment: This patient developed acute tuberculous pericarditis while under observation. A two weeks' course of streptomycin was started about two weeks after the onset of the pericarditis. This treatment had no apparent effect on the pericarditis although it was followed by dramatic improvement in the general status. Signs of pericardial compression appeared, and operation under the protection of streptomycin was considered seriously.

Unfortunately, the tuberculosis disseminated widely under observation. This case suggests, that since pericarditis is often the first serious clinical indication of widespread lymphohematogenous tuberculosis (11), treatment with streptomycin should be continued over a longer period than two weeks early in the disease.

J. S., a 39 year old Porto Rican man entered the hospital November 16, 1949 with a diagnosis of tuberculous pericarditis.

A diagnosis of pulmonary tuberculosis had first been made in June, 1948. In December, 1948 there was an infiltration of the middle third of the right lung demonstrated by X-ray, and the sputum was positive for acid fast organisms. In June, 1949, the pericardial shadow was noted by X-ray to be enlarging. The venous pressure and circulation time were normal. The electrocardiogram showed low voltage with inversion of T waves in Leads 1 and 2. A diagnosis of tuberculous pericarditis was made and streptomycin 1 Gm. daily was started on June 13th and continued for 6 weeks. Between May and July the temperature ranged between 102 and 103°. On June 27th the liver was felt 6 cm. below the right costal margin. A paradoxical pulse was noted. On July 8th, a pericardial tap yielded 100 cc. of bloody fluid which was negative for acid fast bacilli on culture. On August 29th, the liver was enlarged to 10 cm. below the costal margin. Bilateral pleural effusions appeared early in September, and ascites was found late in September. At this time the patient first complained of orthopnea. Edema of the ankle appeared in mid-October. Treatment with Mercupurine was helpful in reducing the edema. Digitalis was ineffective. From July to September the temperature was between 100 and 101°. Because of the signs of increasing pericardial constriction, the patient was referred to Bellevue.

On admission on November 16, 1949 the temperature was 99.4°F., the respirations were 34 and the pulse was 102. The systolic blood pressure was 106; diastolic pressure could not be determined. There was orthopnea, cyanosis and dyspnea. There was dullness with suppression of breath sounds at both bases posteriorly, rising in the axillary regions. The area of precordial dullness extended to the left mid-axillary line. No precordial pulsation was seen. The heart sounds were distant. There was moderate ascites but no ankle or sacral edema. The sputum was negative for acid fast bacilli.

The patient was put on a salt-low diet and given frequent injections of mercupurine. Bilateral chest taps and an abdominal paracentesis produced marked relief. Fluids removed from the chest and abdomen were sterile on cultures for acid fast bacilli. He remained afebrile and improved greatly.

On February 10, 1950, a pericardectomy was carried out. A plane of cleavage was found separating the epicardium from the pericardium. The dissection was carried out completely around the heart, and along the superior and inferior vena cavae. Because the heart showed a tendency to dilate during operation, all of the pericardium was not removed, but a vent about 8 cm. by 6 cm. was left over the anterior surface of the heart.

Following operation, there was a fall in the venous pressure to 130 mm. of saline. There was no reaccumulation of pleural or abdominal fluid. Prolonged bed rest and observation was recommended when the patient was transferred to a sanitarium.

Comment: This patient was given streptomycin shortly after the diagnosis of acute tuberculous pericarditis had been made. After a prolonged course of streptomycin, the temperature returned to normal. The original lung lesion disappeared by X-ray. When signs of constrictive pericarditis did appear, operation could be carried out because both the lung and pericardial lesions were quiescent. Streptomycin, although given early, did not prevent the development of constrictive pericarditis. The principal effect of streptomycin in this patient was to control the pulmonary tuberculosis and to allow early operation.

Despite the apparent good early results obtained by streptomycin and surgery in such patients, it cannot be recommended too strongly that they be continued for long periods after operation on bed rest and streptomycin.

R. Be., a 29 year old colored female entered Bellevue Hospital on June 6, 1949 with a diagnosis of tuberculous pericarditis. The illness had started in December, 1948, with intermittent fever. In January, 1949 at another hospital, there was a high intermittent fever, with a greatly enlarged heart shadow demonstrated by X-ray and distant heart sounds. Exertional dyspnea followed, and two pericardial taps were done yielding large amounts of fluid, from which no acid fast bacilli were obtained. Late in January, the liver was found to be enlarged; ascites formed and gradually increased. In February, 1949, a two weeks' course of streptomycin was given without apparent benefit. Fever continued, and there was increasing ascites. A second course of streptomycin was started on May 5, 1949, and continued until transfer to Bellevue. Again there was no apparent effect from the streptomycin.

When seen at Bellevue on June 6, 1949, the temperature was 99.4°F., the pulse 130, the respirations 22, and blood pressure 110/90. There was evident loss of weight. A bilateral pleural effusion was present. There was distention of the cervical veins. The patient was not orthopneic, but felt better when lying on her right side. No cardiac impulse could be seen or palpated on the chest wall. The area of cardiac dullness extended to the left mid-axillary line. The heart sounds were distant, the second pulmonic sound was slightly louder than the second aortic sound. No friction rub was heard. The liver was felt 5 cm. below the right costal margin. There was shifting dullness in the abdomen.

Thoracenteses on both sides, and an abdominal paracentesis were done. Cultures were negative for acid fast bacilli. Pericardial taps were done shortly after admis-

sion, yielding several hundred cc. of fluid each time. The pericardial fluid of August 4th was positive for acid fast organisms on culture.

A third course of streptomycin 1.5 Gm. daily was started on July 15th. Within several weeks the temperature returned to normal. Signs of ascites gradually increased. On July 5th, a paradoxical pulse was noted for the first time. The temperature ranged between 100 and 101° F. from admission to September, 1949. From then until February, 1950 it was generally between 99 and 100° F. The heart shadow slowly decreased in size.

On March 31, 1950, a pericardectomy was done, and the thickened pericardium over the anterior surfaces of the heart was removed. Examination of the material removed at operation showed a thick layer of dense hyalinized fibrous tissue with areas of granulation tissue. Throughout these tissues there was slight diffuse and focal infiltration of lymphocytes. No tuberculous lesions were seen. Following operation there was a gradual decrease in the ascites, and the liver became smaller. Streptomycin was given until the patient was transferred to a sanitarium in August, 1950. Prolonged rest and observation was recommended.

Comment: The outstanding feature of this case was the persistence of fever and the finding of tubercle bacilli in the pericardial effusion despite two courses of streptomycin, the first one obviously too short to be effective. Despite the ineffectiveness of the streptomycin in sterilizing the pericardial effusion, it did cause some drop in temperature after the beginning of the third course. Again, the use of streptomycin made possible early operation and relief of the symptoms of pericardial constriction.

Although no tuberculous tissue was found in the material removed at operation, it is probable that active tuberculous lesions remained at the time of operation. For this reason a period of bed rest for one year with continued streptomycin treatment was advised on transfer to a sanitarium.

L. W., a 48 year old colored man came to The Johns Hopkins Hospital on June 30, 1950, complaining of pain in the side and fluid on the chest for 6 months.

The patient had been well until October 21, 1949 when cough with fever and a small amount of mucoid sputum appeared. Several days later, there was an episode of weakness, dizziness and sweating followed by sudden right lateral pleural pain. On the following day there was sharp pain in the left lower chest. Several days later the pain grew worse and was associated with dyspnea. At another hospital a right thoracentesis was done yielding 250 cc. of clear fluid, negative on culture for tubercle bacilli. Thereafter he improved and was discharged.

In February, 1950 slight swelling of the face in the morning and swelling of the ankles at night occurred. Dyspnea and orthopnea gradually increased, followed by swelling of the abdomen. In another hospital abdominal paracenteses and

repeated right thoracenteses brought temporary relief. Dihydrostreptomycin 1 Gm. daily with 12 Gm. of para-amino salicylic acid was started and continued for 6 weeks with little change in the patient's symptoms. He was sent to The Johns Hopkins Hospital for further care.

On examination on June 30, 1950 the temperature was 100° F., the pulse 88, respirations 18 and the blood pressure 124/92. There was pulsus paradoxus. There was slight pitting edema of the face and ankles. The cervical veins were distended. The left chest was clear to examination, but on the right side, there were signs of fluid up to the scapular spine. There was no visible or palpable point of maximal cardiac impulse. The cardiac dullness extended 12 cm. to the left of the mid-sternal line in the fifth interspace. The heart sounds were distant; the second pulmonic sound was louder than the second aortic sound, and no friction rub was heard. The liver edge was palpable 6 cm. below the right costal margin. There was no ascites.

There was no anemia or leucocytosis. The sedimentation rate was 25 mm. in one hour corrected by the Wintrobe method, and remained at this level during the stay in the hospital. The venous pressure was 325 mm. of saline in the arms and in the legs. The circulation time was 45 seconds with decholin. The X-ray of the chest showed a massive pleural effusion on the right and kymography demonstrated diminution of the pulsations of the heart. The temperature was normal after the first hospital day.

Repeated right thoracenteses were done. Sputum and pleural fluid cultures remained negative for tubercle bacilli. On a regimen of salt restriction and mercurial diuretics, there was a gradual loss of weight. On July 14 the daily administration of 1 Gm. of streptomycin was started and continued until September third.

A diagnosis of tuberculous pericarditis was made, and, on July 19th, a pericardectomy was done through a median sternotomy. The pericardium was thickened and was stripped from the anterior surface of the heart. The portions of the pericardium removed at operation showed Langhan's giant cells with macrophages and epitheloid cells. There was extensive scar tissue around the tubercles, with little caseation. During the postoperative period there was bilateral accumulation of pleural fluid, right bundle branch block, and continued elevation of the venous pressure. Early in August the bundle branch block disappeared, the venous pressure dropped, and the excessive formation of pleural fluid ceased. By early September, he felt perfectly well, was afebrile and had no signs of generalized tuberculosis or of constrictive pericarditis. At discharge, prolonged bed rest at home was advised.

Comment: Operation was carried out six months after the first symptoms of constrictive pericarditis. The removed pericardium showed evidence of tuberculosis with scar formation. It is probable

that treatment with streptomycin made possible early operation and prevented spread of the tuberculosis. However, the presence of tubercles in the material removed at operation shows that streptomycin had by no means sterilized the pericardium, and that prolonged bed rest was necessary after operation.

DISCUSSION

Tuberculous pericarditis differs from tuberculosis elsewhere in the body in that the actual healing process may cause interruption of physiological processes by constriction. Furthermore, healing and scarring may prevent high concentrations of streptomycin from reaching the tubercle bacilli. Early treatment is therefore extremely important to facilitate penetration of streptomycin into the tissues and healing with as little scarring as possible.

Because early treatment is so important, it may frequently be necessary to administer streptomycin before the diagnosis is proved. Isolation of tubercle bacillus from pericardial fluid is extremely difficult even in some cases later proved at autopsy to have tuberculous pericarditis. In many patients, however, the diagnosis may be strongly suspected from the course of the disease or from the isolation of the tubercle bacillus from sources other than the pericardium. The cases which give the most difficulty are those in which the tuberculous process is clinically limited to the pericardium, and in which the pericardial effusion is small or in which tubercle bacilli can not be demonstrated in smear and in culture. Because pericarditis may be the first sign of widespread tuberculosis (11), because streptomycin is probably more effective early than late, and because little harm can be done by a course of streptomycin, we believe that it should be administered early in questionable cases after all diagnostic procedures have been exhausted. Once started, the streptomycin therapy should be continued for several months (12) in conjunction with para-amino-salicylic acid (13), (14) even though no dramatic change in the course of the disease occurs within several weeks.

Occasionally patients with widespread tuberculosis may have increasingly severe symptoms of constrictive pericarditis. The use of streptomycin may promote general improvement so that operation can

be undertaken. Following operation streptomycin and para-aminosalicylic acid should be continued for a prolonged period to prevent spread of infection and local abscess formation. Bed rest after operation should not be neglected even though signs of constrictive pericarditis have improved.

SUMMARY

An analysis of the long time course of symptomatic tuberculous pericarditis in 71 patients showed that 40.5 per cent died of widespread tuberculosis, 8.5 per cent died of cardiac failure, 19.5 per cent survived for 5 years or more. The fate of the remainder is unknown. Of 21 patients alive 5 years or more after the original pericarditis, only three had any signs or symptoms attributable to previous tuberculous pericarditis.

In tuberculous pericarditis, streptomycin appears to be particularly useful to control or to prevent dissemination of tuberculosis. It is of great importance to start streptomycin therapy early in suspected cases of tuberculous pericarditis after all diagnostic procedures have been performed. Streptomycin is helpful in preparing patients for pericardectomy. The beneficial effect of prolonged bed rest and streptomycin after surgery should not be overlooked even though the symptoms of constrictive pericarditis have been relieved by operation.

The author wishes to thank Drs. J. Burns Amberson, Jr., A. McGehee Harvey, E. Cowles Andrus and Elliot V. Newman for helpful suggestions and criticism.

BIBLIOGRAPHY

1. Meredith, H. Clarkson Jr.: Streptomycin in Acute Tuberculous Pericarditis. Am. Heart J. **37:** 192, 1949.
2. Holman, Emile and Willett, Forest: The Surgical Correction of Constrictive Pericarditis. S.G. & O. **89:** 1, 1949.
3. Topie, J., Laporte, J., Pinel and Monnier: Effect of Streptomycin in Treatment of Tuberculous Pericarditis. Presse Med. **58:** 226, March 4, 1950.
4. Johnson, Richard P. and Bercu, Bernard A.: Streptomycin in the Treatment of Tuberculous Pericarditis. Am. Rev. Tuber. **59:** 656, 1949.
5. Harvey, A. M. and Whitehill, M. R.: Tuberculous Pericarditis. Medicine **16:** 45, 1937.
6. Blalock, Alfred and Levy, Sanford: Tuberculous Pericarditis. J. Thoracic Surg. **7:** 132, 1937.
7. Churchill, E. D. in discussion of above paper.

8. PEEL, A. A. FITZGERALD: Tuberculous Pericarditis. Brit. Heart Journal **10:** 195, 1948.
9. DIEFENBACH, WILLIAM: Tuberculosis of the Heart, A Review. Am. Rev. Tuberc. **62:** 390, 1950.
10. AUERBACH, OSCAR: Pleural, Peritoneal and Pericardial Tuberculosis: A Review of 209 Cases Uncomplicated by Treatment of Secondary Infection. Am. Rev. Tuberc. **61:** 845, 1950.
11. GOLDSTEIN, J.: Tuberculous Pericarditis as a Forerunner of Generalized Miliary Tuberculosis. Tubercle **30:** 14, 1949.
12. TEMPEL, CARL W. AND DYE, WILLIAM E.: Selecting the Streptomycin Regimen for Patients with Pulmonary Tuberculosis with Special Reference to the Intermittent Dosage Schedule. Dis. of Chest, **16:** 704, 1950.
13. DUNNER, EDWARD, BROWN, WALTER AND WALLACE, JACK: The Effect of Streptomycin with Para-Amino Salicylic Acid on the Emergence of Resistant Strains of Tubercle Bacilli. Dis. of Chest **16:** 660, 1950.
14. TEMPLE, CARL W., HUGHES, FREDERIC J., MARDIS, RICHARD E., TOWBIN, MILTON N. AND DYE, WILLIAM E.: Combined Intermittent Regimens Employing Steptomycin and Para-aminosalicylic Acid in the Treatment of Pulmonary Tuberculosis. Transactions of the Forty-Sixth Annual Meeting of the National Tuberculosis Association, p. 27, 1950.

THE PATHOLOGY OF THE OSSEOUS CHANGES IN CUSHING'S SYNDROME IN AN INFANT AND IN ADULTS

RICHARD H. FOLLIS, JR.

Department of Pathology, The Johns Hopkins University School of Medicine

Received for publication January 25, 1951.

Since Cushing's (1,2) delineation of the syndrome which bears his name, skeletal changes have been a prominent part of the total picture. In children, stunting of growth is commonly seen; while in adults, osteoporosis is a prominent feature. Both of these manifestations of bone disease have been observed time and time again clinically and roentgenographically. However, scant, or no attention has been directed at the pathologic anatomy of these disturbances. After reviewing the reports of cases of Cushing's syndrome which have come to autopsy one is surprised to find that with few exceptions only superficial examination seems to have been made of the skeletal tissues. Since we have recently had an opportunity to study material from 5 cases of Cushing's syndrome (one infant, four adults), we have felt that it was desirable to record the microscopic findings in the bones. This was particularly true in the case of the infant, since in our experience the changes which were encountered are unique.

CASE REPORTS

Case 1 (19088). A white male infant 15½ months of age at the time of death was first admitted to the Harriet Lane Home 4 months before because of diarrhea and polydypsia. On examination a tumor was found in the left chest; on exploration this proved to be a benign ganglioneuroma. He was sent home to come back for a lobectomy in 4 months. On return his appearance had changed most dramatically; he was obese, hirsute, had developed comedones on the face and had a thin skin. There were no striae; the genitalia were normal. Exploratory laparotomy revealed "normal adrenals and kidneys." He died following this procedure.

At autopsy the external appearance noted above was confirmed. The subcutaneous fat of the abdomen and thorax was greatly increased. The heart was slightly enlarged (80 grams). The liver was enlarged, weighing 600 grams (normal for this age 330 grams). Both adrenals were enlarged resembling those of an adult in size. The thymus appeared to be replaced by fat.

Microscopically the pertinent findings were as follows: The hypophysis was

not enlarged; in the area between the anterior and posterior lobes there were cord like structures made up of basophilic staining cells arranged in a sort of papillomatous yet diffuse fashion. There was no distinct adenoma. Both adrenals, as noted above, were enlarged; this was due to an increase in width of cortical cells. The zona glomerulosa was prominent as were the fasicular and reticular layers. The thymus could only be identified as such by the Hassal corpuscles which were found imbedded in fat. The spleen was extraordinary in that it was devoid of Malpighian bodies.

Several costochondral junctions were available for study (Figures 2 and 4). Here the resting cartilage cells were normal in appearance. However, as one approached the cartilage-shaft junction the usual orderly arrangement of the cells into rows was not found. Instead, the cells were disorganized and arranged in a completely disordered manner. The intercellular cartilage matrix was increased in amount. In addition, although there were occasional foci of lime salt deposition, these were scanty and no regular deposition of inorganic material in columns at the junction with the shaft was found . At the costochondal junction the cartilage was convex, the shaft concave. There were no hypertrophic cells lined up to be invaded by capillaries. Most of the undersurface of the cartilage was covered by bone. What few trabeculae there were just beneath the cartilage contained no cores of calcified matrix, such as one finds normally. Many of the trabeculae present were covered by thin borders of pink staining osteoid. No osteoclasts were in evidence though a fair number of normal appearing osteoblasts were found about the trabeculae. As noted, there was no vascular invasion of the cartilage whatsoever. In the shaft the rarefied trabecular pattern was most disorganized and irregular. Some of these structures also had thin osteoid borders (Figure 14). The distribution of the osteocytes in the trabeculae seemed normal. No osteoclasts were seen. There was some periosteal new bone formation. Although the marrow elements were fairly abundant, one had the definite impression that an increase in the fat content of the medullary cavity was present.

In summary this was a 15½ months old male infant who at the time of death displayed evidences of Cushing's syndrome and who at autopsy was found to have localized accumulations of basophilic cells in the hypophysis, adrenal hyperplasia, complete loss of lymphocytes from the thymus and spleen and a chondodystrophic type of lesion of the ribs with disturbance in calcification in the cartilage and rarefaction of the shaft. We shall discuss certain features of this case below.

Case 2 (13960). This case has been reported in detail elsewhere by MacCallum et al. (3). A white female 25 years of age had been well until one year before when she noted gain in weight, cessation of menstruation and change in facial appearance. On examination there was plethora, obesity of the face and trunk, striae and

hypertension. Roentgenograms of the skeleton showed rarefaction. In order to study the adrenals retrograde pyelograms were taken on the right side. Following this procedure her temperature rose; she developed septicemia and died.

Autopsy confirmed the external appearance. The adrenals were slightly enlarged, the left one weighing 14.7 grams. There was a large basophil adenoma in the hypophysis.

The skull and vertebra were available for microscopic study (Figure 6). The trabeculae were extremely thin in comparison with a control group, a series reported elsewhere (4) of individuals dying of trauma of short duration. There were osteoblasts about the trabeculae; the osteocyte pattern in these thinned structures was normal. No osteoclasts were present nor were there any osteoid borders. The content of medullary fat appeared increased.

In summary this is a typical case of Cushing's syndrome associated with a basophilic adenoma of the hypophysis. Microscopically the bones showed extreme rarefaction with no explanation to account for this change.

Case 3 (18626). A 20 year old white female had been normal until 2½ years before coming to the hospital. At this time she began to gain weight and developed excessive hair on the face, back and extremities. Amenorrhea also occurred. Because of her worsening condition she took an ounce of formaldehyde in an unsuccessful attempt at suicide. Following this episode she developed a lung abscess which was operatively drained. On examination there was plethora, buffalo type obesity, moon face, hypertrichosis, long red striae and hypertension. While undergoing various examinations in the Diagnostic Clinic she developed severe headache and lapsed into coma. Her temperature rose; she developed left sided weakness. Since brain abscess was suspected, she was explored and an abscess was evacuated. She died some hours following this procedure.

At autopsy the heart was enlarged; there were multiple cerebral abscesses. A small (5 mm.) basophilic adenoma was found in the anterior lobe of the hypophysis. There were prominent reticular zones in both adrenal glands.

Specimens of the vertebra were available for study. Microscopically, these showed (Figure 7) extremely thin trabeculae surrounded by normally appearing osteoblasts. The osteocyte pattern in these trabeculae seemed normal. However, osteoid borders were present about many of the trabeculae. Such borders were not wide but were considered abnormal (Figures 11 and 12). No osteoclasts were seen.

In summary, this was a typical instance of Cushing's syndrome associated with a basophilic tumor of the hypophysis. The vertebra exhibited extreme rarefaction with thin trabeculae. Osteoblasts were numerous; thin borders of osteoid were found about the trabeculae.

Case 4 (19463). A 33 year old white female came to the outpatient clinic complaining of swelling of the face, hands and ankles of one years duration. Following this she noted an increase of hair on her face and cessation of menstruation. Examination revealed a plethoric woman with girdle type obesity, rounded face, hirsutism, hypertension and glycosuria. X-ray examination revealed osteoporosis with beginning collapse of the body of the 11th thoracic vertebra. Since a mass was thought to be displacing the left kidney, the patient was explored and a tumor 8 cm. in diameter was removed from the region of the left adrenal gland. She did poorly following the operation and despite supportive measures, including cortical extract, died three days later. The tumor was composed of highly differentiated cells resembling those of the adrenal cortex.

At autopsy a small metastasis of tumor was found in the left lung. The right adrenal was decidedly atrophic. There were hyaline basophiles in the hypophysis.

Specimens of vertebra were available for microscopic study (Figure 8). The trabeculae were greatly thinned. Osteoblasts appeared to be present in normal numbers as were osteocytes in the trabeculae. Borders of osteoid were found about the trabeculae but this material was by no means excessive (Figure 13). No osteoclasts were present.

In summary this is an instance of typical Cushing's syndrome associated with a carcinoma of the adrenal cortex. Microscopically, the trabeculae in the vertebrae were greatly thinned. Otherwise no abnormal changes were observed.

Case 5 (S.P. 50-3089). A 28 year old white female was first seen with the complaint of increasing appetite and weight gain of 2 years duration. About 6 months later she began to notice the development of acne, hirsutism, deepening of the voice and puffiness of the face. Amenorrhea also developed. Physical examination confirmed these complaints and also revealed hypertension and an increased excretion of 17-Ketosteroids. She was treated by x-radiation to the pituitary with no apparent improvement. She had 2 other hospital admissions during an ensuing 8 months period. The present admission was because of the sudden appearance of severe right chest pain; this was interpreted as being due to a fractured rib. As her general condition was no better it was decided to explore the adrenals. At operation a subtotal removal of the right adrenal was performed. The specimen removed showed hyperplasia. A specimen of rib in the region of the fracture was also obtained. Inasmuch as the clinical diagnosis of Cushing's syndrome has been established we do not feel it necessary to comment on her subsequent course.

Microscopically the shaft of the rib (Figure 9) away from the region of fracture showed great rarefaction of the cortex and virtually no medullary trabeculae. Some which were present had thin borders of osteoid about them. Osteoplasts were plentiful. No osteoclasts were in evidence. In the region of the fracture it was obvious that some reparative ability was present, since sheets of pink staining osteoid

were found about fragments of disorganized trabeculae (Figure 10). Osteoblasts were present in such areas. Much of this material was not impregnated with inorganic material.

In summary this is a typical clinical case of Cushing's syndrome. The rib showed great rarefaction. About the fracture there was evidence of repair.

DISCUSSION

In describing the syndrome which bears his name, Cushing called attention to involvement of the skeleton, noting in his first report (1) before The Johns Hopkins Medical Society that in six of the eight autopsied cases there was "an osteoporosis of the skeleton most marked in the spine". A year later in his Harvey Lecture (2) he referred to "skeletal decalcification" and employed the term "osteomalacia" in describing the skeletal changes. The terms osteoporosis and osteomalacia, of course, connote two entirely different processes. The former refers to bone disease arising from decreased osteoblastic activity as evidenced by thinned trabeculae which, however, are normally impregnated with inorganic elements, principally calcium, phosphate and carbonate. On the other hand osteomalacia, the adult counterpart of rickets, is characterized by normal, or even increased, osteoblastic activity; the organic matrix (osteoid) which is produced, however, is deficient in its complement of inorganic elements. These two diseases may, of course, coexist.

Few detailed studies of the bones of adults coming to autopsy with the characteristic generalized changes of Cushing's syndrome have been reported. With respect to infants and children we have not been able to find any adequate studies.

In our experience, based on examinations in collaboration with Dr. E. A. Park of bones, particularly the costochondral junctions, from autopsies on over 1500 infants and children (5,6), the changes found in the 15½ months old infant (Case 1) described above are unique, and do not resemble changes which may be encountered in the cartilage under a variety of circumstances: hypopituitarism, hypothyroidism, non-specific inanition, excessive x-ray irradiation, rickets, hereditary achrondroplasia and gargoylism (Hurler's syndrome). We have not had an opportunity to examine the hypopituitary human;

in animals there is merely a slowing of the normal orderly growth sequences as Evans and his colleagues (7) have shown. So too, the bones of the cretin exhibit a somewhat similar pattern at the costochondral junction, i.e. a retardation in normal growth sequences. In addition however, there are rather large quantities of calcified cartilagenous matrix encased in bone deep in the shaft in children dying in the hypothyroid state. Inanition, whether occurring in man or experimental animals, leads to cessation of growth of both cartilage and bone. One finds, however, an orderly appearance of the cartilage in that the pattern of row formation persists, even though the under surface may be covered by a layer of bone. X-ray irradiation (17) leads to profound changes in the epiphyseal cartilage of experimental animals with marked disorganization and arrest of cartilage growth sequences. However, the underlying bone of the shaft exhibits sclerosis. In rickets hypertrophic cartilage cells are always found even though the disorganization at the costochondral junction may be extreme. So too, there is always some invasion of the cartilage by capillaries. In advanced cases of rickets an accentuation of osteoblastic activity is everywhere present. In the chondrodystrophic dwarf the cartilage cells mature, though slowly, and inorganic materials are deposited in normal fashion. Here the primary disturbance appears to be a retardation in the rate of growth and maturation of the cartilage cells. A similar change is encountered in gargoylism; in this syndrome rather characteristic systemic changes are also found.

It is apparent that the lesions in Case 1 in no way resemble those which have just been enumerated. We do not feel that the disturbances in calcification of the cartilage and of the trabeculae in the shaft were due to rickets although at this infants' age rickets is not at all uncommon (8). However, it is our opinion that the primary alteration of the cartilage and bone is a disturbance in chondrogenesis and osteogenesis. In view of the current interest in the rôle of the adrenal in bodily processes particularly with respect to Cushing's syndrome, it is of interest that in 1944, Becks et al. (9) reported that when ACTH was administered to rats there was retardation in the growth of cartilage; their illustrations are reminiscent of changes encountered in hypophysectomized animals or as a result of inanition. Baker and Ingle (10) have also reported on the skeletal effects of ACTH, noting nar-

rowing of the epiphyseal cartilage of the tibia, atrophy of osteoblasts and reduction in osteoclasts. Wells and Kendall (11) studied the effects of Compound E in rats, noting a retardation of growth; no morphological observations were made. More recently Winter et al. (18) administered 3 mg. cortisone daily to rats weighing 200 grams. Sections of the ribs and tibiae revealed a reduction in the width of the epiphyseal cartilage but no rarefaction of the cortical or cancellous bone so that the picture encountered in human cases of Cushing's syndrome was not reproduced.

Anatomical evidence of disturbance in the growth of cartilage is, of course, to be expected in cases of Cushing's syndrome in children and adolescents. Shortly after Cushing's reports, Freyberg and Grant (12) described the persistence of epiphyses which should have become closed in a male aged 19 years; the x-ray age was "15 years or less". Both Albright (13) and Wilkins (14) have drawn attention to the retardation of growth in children and adolescents with Cushing's syndrome.

We shall not review the scanty literature on the pathologic anatomy of the skeletal lesions in adults. Mooser (15) whose anatomical description furnished Case 9 to Cushing's series (1) described the bone lesions in detail, noting that the change observed was not osteomalacia or late rickets. He also reported the absence of osteoclastic resorption. In 1933, Rutishauser (16) studied in some detail the osseous changes of 2 cases noting that the disturbance was osteoporosis. These early reports are the most complete which have come to our attention.

From a purely morphological study of the 4 adults described above one gains little or no insight as to the underlying disturbance. Osteoblasts are present and appear to be competent to produce the organic matrix of bone osteoid as evidenced by the presence of thin osteoid borders about many trabeculae and the healing process in Case 5. There is no evidence of any excess destruction whatsoever. We remain completely in the dark at this time and in our ignorance it seems unwise to speculate on the pathogenesis.

SUMMARY

Five cases of Cushing's syndrome have been reviewed from the standpoint of skeletal lesions. The changes in the rib of a 15½ month

old infant consisted of a peculiar arrest in growth and disorganization of the cartilage with rarefaction of the shaft all of a type not heretofore observed. The vertebra and ribs of 4 adults showed rarefaction of the trabeculae with seemingly adequate numbers of osteoblasts and no evidence of osteoclastic resorbtion.

BIBLIOGRAPHY

1. Cushing, H., The Basophil Adenomas of the Pituitary Body and Their Clinical Manifestations (Pituitary Basophilism), Bull. Johns Hopkins Hosp., 1932, **50**, 137.
2. Cushing, H., "Dyspituitarism": Twenty Years Later with Special Considerations of the Pituitary Adenomas, Harvey Lectures, 1932–1933, Williams and Wilkins Company, Baltimore, p. 90.
3. MacCallum, W. G. Futcher, T. B., Duff, G. L., and Ellsworth, R., Relation of the Cushing Syndrome to the Pars Intermedia of the Hypophysis, Bull. Johns Hopkins Hosp., 1935, **56**, 350.
4. Follis, R. H., Jr., and Jackson, D., Renal Osteomalacia and Osteitis Fibrosa in Adults, Bull. Johns Hopkins Hosp., 1943, **12**, 232.
5. Follis, R. H., Jr., Park, E. A. and Jackson, D., The Prevalence of Scurvy at Autopsy During the First Two Years of Age, Bull. Johns Hopkins Hosp., 1950, **87**, 569.
6. Follis, R. H., Jr., Jackson, D. A., Eliot, M. M., and Park, E. A., Prevalence of Rickets in Children Between Two and Fourteen Years of Age, Am. J. Dis. Child., 1943, **66**, 1.
7. Simpson, M. E., Arling, C. W., and Evans, H. M., Some Endocrine Influences on Skeletal Growth and Differentiation, Yale J. Biol. and Med., 1950, **23**, 1.
8. Follis, R. H., Jr., Park, E. A., and Jackson, D. A., The Prevalence of Rickets at Autopsy During the First Two Years of Age, Bull. Johns Hopkins Hosp. To be published.
9. Becks, H., Simpson, M. E., Li, C. H., and Evans, H. M., Effects of Adrenocorticotropic Hormone (ACTH) on the Osseous System in Normal Rats, Endocrinology, 1948, **43**, 422.
10. Baker, B. L., and Ingle, D. J., Growth Inhibition in Bone and Bone Marrow Following Treatment with Adrenocorticotropin (ACTH), Endocrinology, 1948, **43**, 422.
11. Wells, B. B., and Kendall, E., The Influence of Corticosterone and C_{17} Hydroxydehydrocorticosterone (Compound E) on Somatic Growth, Proc. Staff Meetings Mayo Clin., 1940, **15**, 324.
12. Freyberg, R. H., and Grant, R. L., Calcium and Phosphorus Metabolism in a Verified Case of Pituitary Basophilism. Arch. Int. Med., 1936, **58**, 213.
13. Albright, F., Cushing's Syndrome: Its Pathological Physiology; its Relation-

ship to the Adreno-genital Syndrome and its Connection with the Problem of the Reaction of the Body to Injurious Agents ("Alarm Reaction" of Selye), Harvey Lecture Series, 1942–43, **38,** 123.

14. WILKINS, L., The Diagnosis and Treatment of Endocine Disorders in Childhood and Adolescence, 1950, Charles C. Thomas, Publisher, Springfield.
15. MOOSER, H., Ein Fall von endogenes Fettsucht mit hoch gradiger Osteoporose. Virch. Arch., 1921, **229,** 247.
16. RUTISHAUSER, E., Osteoporotische Fettsucht, Deut. Arch. f. Klin. Med., 1933, **175,** 640.
17. GALL, E. A., LINKLEY, J. R., AND HILCKEN, J. A., Comparative Experimental Studies of 200 Kilovolt and 1000 Kilovolt Roentgen Rays. I The Biological Effects on the Epiphysis of the Albino Rat, Am. J. Path., 1940, **16,** 605.
18. WINTER, C. A., SILBER, R. H., AND STOERK, H. C., Production of Reversible Hyperadrenocortinism in Rats by Prolonged Administration of Cortisone, Endocrinol., 1950, **47,** 60.

PLATES

PLATE I

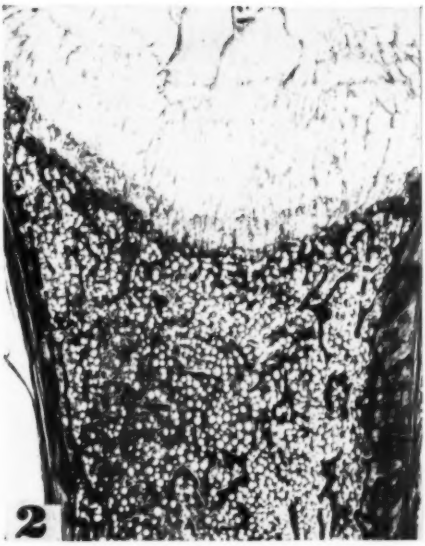
FIG. 1. Low power magnification of normal costochondral junction of a 16 months old child dying acutely of meningitis. Note straight line of ossification and fairly dense trabecular pattern in shaft.

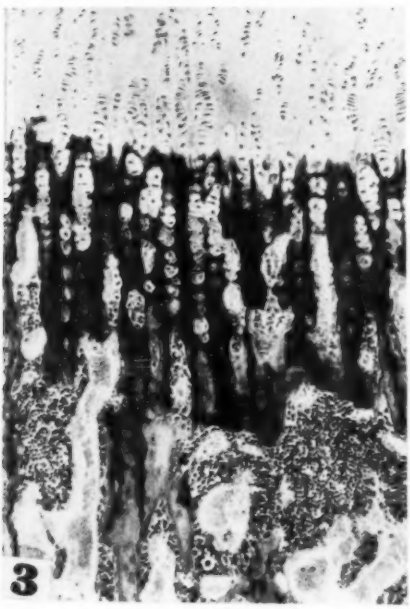
FIG. 2. Low power magnification (same as Figure 1) of costochondral junction from Case 1. Note concave shaft and convex cartilage and irregular trabecular pattern.

FIG. 3. Higher power of costochondral junction seen in Figure 1. Note regular rows of cartilage cells with abundant calcified matrix in orderly arrangement being encased by bone.

FIG. 4. Case 1. Note peculiar irregular calcification of irregularly disposed cartilage cells, virtual absence of any calcified matrix lattice and relative absence of any bone below.

1


2


3

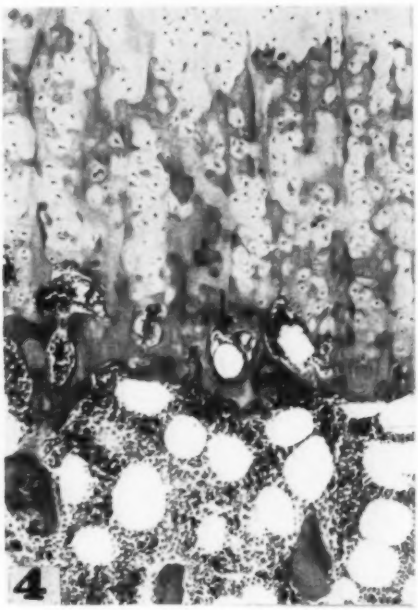

4

PLATE II

FIG. 5. Low power view of portion of vertebral body from a 34 year old female dying very shortly after fracturing her skull. Note width of trabeculae.

FIG. 6. Case 2. Low power field (same magnification as Figure 5) of vertebral body to show great reduction in width of the trabeculae.

FIG. 7. Case 3. Low power field (same magnification as Figure 5) of vertebral body to show same reduction in width of trabeculae.

FIG. 8. Case 4. Low power field (same magnification as Figure 5) of vertebral body to show identical reduction in width of trabeculae. One had the impression in this section that the number of trabeculae were reduced as well.

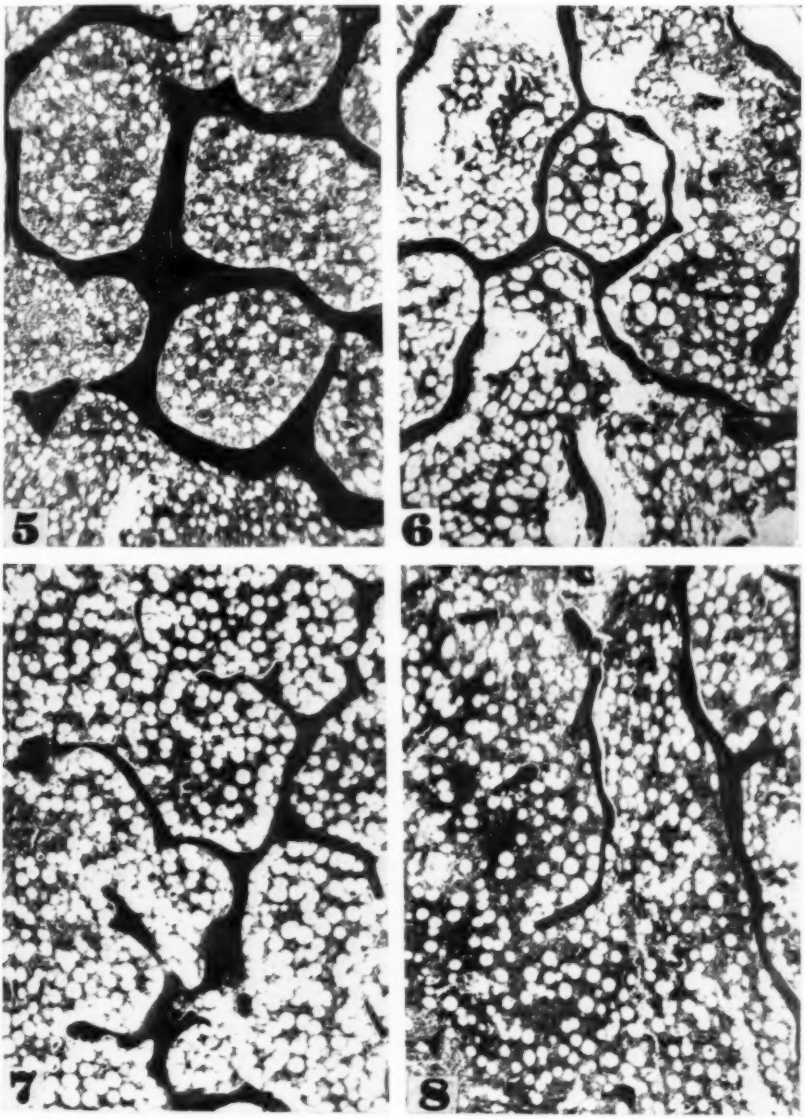

5
6
7
8

PLATE III

FIG. 9. Case 5. Low power field of cortex and medullary cavity (same magnification as Figure 5) to show reduction in width of cortex and thin trabeculae which are reduced in number as well.

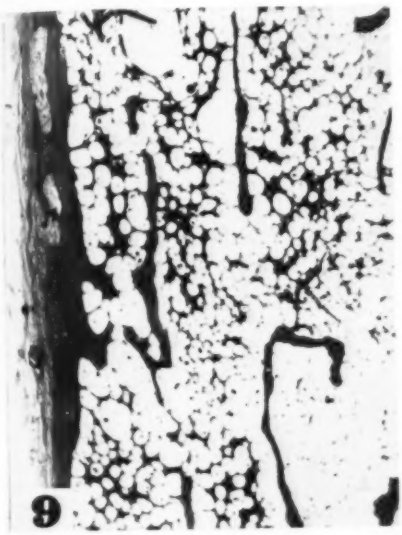
FIG. 10. Case 5. High power field of area of fracture which shows several bony fragments surrounded by osteoblasts which appear to have produced osteoid together with a fibrillar pink staining material.

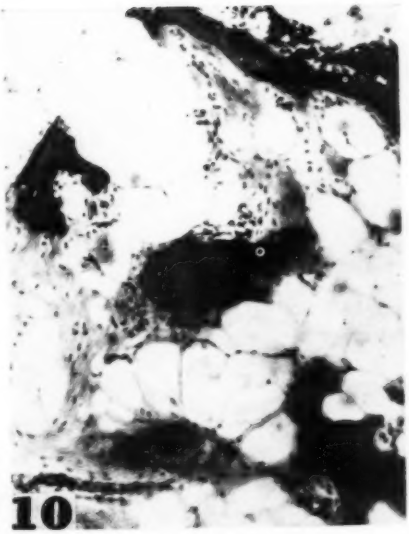
FIG. 11. Case 3. High power magnification to show osteoid border along a trabecula.

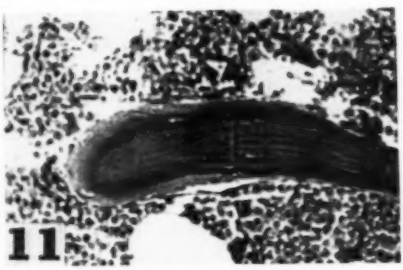
FIG. 12. Case 3. High power magnification to shows osteoid border along trabecula and numerous osteoblasts.

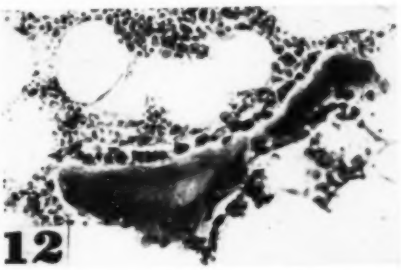
FIG. 13. Case 4. High power magnification to show narrow osteoid border along trabecula and numerous osteoblasts.

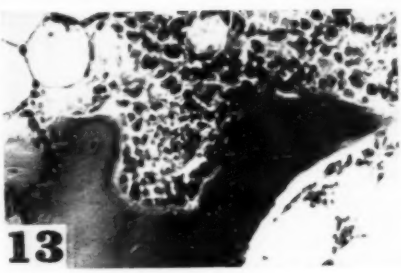
FIG. 14. Case 1. Higher power magnification to show osteoid border along trabecula.

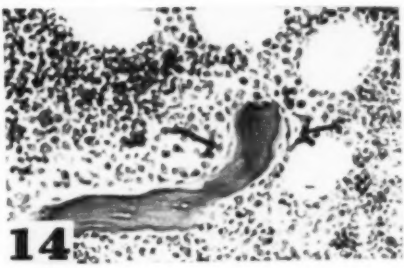

9


10


11


12


13


14

MODIFIED ONE-STAGE HEPATECTOMY IN THE DOG

With Some Notes on the Effect of Hepatectomy on the Coagulability and Proteolytic Activity of the Blood*

RICHARD C. CLAY† and OSCAR D. RATNOFF‡

The Surgical Hunterian Laboratory and the Departments of Surgery and Medicine, the Johns Hopkins Hospital and University

Received for publication February 6, 1951

A valuable method for investigating the many recognized and suspected functions of the liver is study of the metabolism of an animal following total hepatectomy. Since the dog is an animal whose physiologic processes are very similar to those of man, and whose size and blood volume permit adequate post-operative studies, most hepatectomy techniques and studies have employed this animal. The difficulty and high mortality of total hepatectomy have however made it a little used method, though it offers, especially in an era of easily accessible isotope tracers, an excellent technique for studying liver function. It is our purpose to describe a relatively simple modification of a previously published one-stage method of hepatectomy, and to record certain incidental observations on the clotting mechanism and plasma proteolytic activity of animals so prepared.

The original attempts to study a hepatectomized dog involved so called "head-thorax" preparations, animals in which all abdominal viscera were removed, and others in which the liver was excluded from the circulation by ligation of vena cava, hepatic veins, and portal vein *en masse*. Such gross procedures introduced so many factors other than hepatectomy that they were never satisfactory. Mann (1) first devised a method of removing the liver from a dog without interrupting permanently either portal or caval circulations. This method required three separate operations. At the first a lateral anastomosis was performed between portal vein and vena cava and the cava ligated

* These studies were conducted under contract with the Office of Naval Research, U. S. Navy

† Now at 404 Ingraham Building, Miami, Florida

‡Now at Mt. Sinai Hospital, Cleveland, Ohio

between the anastomosis and the liver. Three weeks were allowed to elapse for development of collateral circulation through abdominal wall and azygous systems. The resistance of the hepatic capillary bed to the combined caval and portal flows was the stimulus for formation of these collaterals. Following this period, at a second laparotomy, the portal vein was ligated between the anastomosis and the liver, forcing all inferior caval and portal blood through the new collateral channels. After an unspecified time, at a third laparotomy, the common bile duct and hepatic artery were divided, and the liver was excised with the anterior caval wall where the hepatic veins entered. The cava was ligated subphrenically.

Animals thus hepatectomized became rapidly hypoglycemic and died within about 3 to 8 hours after operation. Mann and associates found that intravenous injections of glucose, maltose, or mannose prolonged survival. But inevitably, after 18 to 30 hours, ataxia, twitching, vomiting, coma and death occurred. These signs were unalterable by carbohydrate injections, and apparently were due to some mechanism other than hypoglycemia. Roughly .25 gms. glucose per kilogram of weight per hour were necessary to maintain the blood sugar level.

Most observations on hepatectomized animals have been made following this complicated three stage procedure (2). Mann pointed out one of the objections to the method: the high mortality necessarily entailed in a series of three laparotomies. A second objection is inherent in the method: the long time necessary to complete the procedure with the great postoperative care involved in bringing the animal to the third and significant laparotomy. A third and vital objection stems from the fact that an animal so prepared has lived many weeks with the abnormal hepatic circulation produced by the reverse Eck fistula. Thus the venous return from the alimentary tract has gained ultimate access to the liver only by way of the hepatic artery, after being mixed with the rest of the venous blood in heart and lungs. One can only guess what compensatory adjustments in metabolism may have come about during these weeks of portal interruption to invalidate observations made following the ultimate hepatectomy. For all these reasons a single stage method of hepatectomy was desirable, if not imperative.

Such a method was described by Firor and Stinson (3) in 1929.

Their technique depended upon the fact that flowing blood would not clot in a paraffined glass cannula. They controlled the blood supply of the liver, after dividing the hepatic artery, by occluding temporarily the portal vein and the vena cava just below the diaphragm and just above the left lumboadrenal vein. They then opened the vena cava just posteriorly to the liver and rapidly introduced a saline filled, paraffined glass side-arm cannula (shaped like the one illustrated in this article) into the opening. Momentary relaxation of the untied ligature about the subphrenic cava permitted the cannula to slip through it. Following this the ligature was quickly tied about the cannula. The two posterior openings of the cannula were stoppered during this phase to prevent air embolism, a major cause of failure of this operation. The posterior caval end of the cannula was then unstoppered and slipped into the caval opening and the cava was ligated about it. Finally the portal side-arm was unstoppered and the end of the portal vein was slipped over it and tied. Once these ingenious maneuvers were accomplished, caval and portal circulations had been restored and the liver was excluded from all circulation. The liver with the necessarily included anterior caval wall could then be totally excised without haste. A rather high mortality was inherent in the method despite great skill and teamwork of the operators. Air embolism constituted the greatest single hazard.

After hepatectomy by this method, the animals survived a shorter time than did those reported by Mann, probably evidence that metabolic adjustments during the Eck fistula life occur. It seems quite possible that these may cause observations following three stage hepatectomy to reflect alterations other than removal of the liver. Firor and Stinson reported survivals up to 14 hours, but the usual time was about 6 to 8 hours (4) if glucose were administered. Their animals demonstrated the same hypoglycemic signs as did Mann's, and with glucose administration they recovered temporarily, only to arrive terminally at the same irreversible state as animals hepatectomized in three stages.

This method is difficult and requires great dexterity during cannulation. This probably accounts for the dearth of reports of its use. But Tarver and Reinhardt (5) have recently used this method to study serum protein synthesis in the absence of the liver. They administered

methionine containing radioactive sulfur to hepatectomized dogs and analyzed the serum proteins postmortem. Their dogs survived 2, 3 and 5 hours, but it is not clear whether they died or were sacrificed. This excellent work illustrates the value of a reliable and rapid method of one stage hepatectomy, and the following procedure we think promises the highest percentage of successful operations of this sort. Like

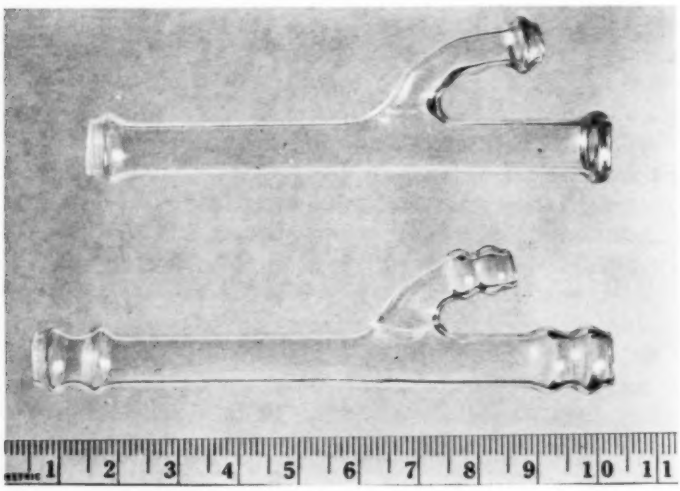


FIG. 1. Pyrex glass side-arm cannulas used to establish portacaval circulation excluding the liver prior to excision of this organ. The long end of the cannula passes through the diaphragm in the vena cava, the short end lies just above the right lumboadrenal vein, while the side-arm receives the portal vein stump. An interior silicone coating prevents clotting in the cannula.

the method of Firor and Stinson, of which the following method is a modification, it depends upon the use of side-arm cannulas. They are illustrated in Fig. 1 which shows their dimensions. We have replaced the paraffin internal coating used by Firor and Stinson with a silicone coating (6). It is relatively permanent and easily applied, sterilizable, and uniformly prevents thrombosis in the cannulas.*

* The cannulas were made by Mr. Harry Eisenberg of the Chemical Division, Department of Medicine of the Johns Hopkins Hospital.

METHOD

A large, healthy mongrel dog in the fasting state is given a hypodermic injection of .01 mg. atropine per kilo of body weight. Morphine is omitted since, after the liver is removed, catabolic destruction of this alkaloid would be impaired and its effect would persist. The shaved area includes the abdomen and right chest to the axilla. The animal is placed supine, anesthetized with ether, intubated, and maintained throughout the procedure on positive pressure insufflation with ether and air. A polythene tube is inserted into a hind leg vein, and a constant slow drip of a mixture of 5% glucose and .85% sodium chloride is started.

The initial incision is that employed by Firor and Stinson: a long upper right rectus curving medially to the xiphoid and extending a little below the umbilicus. It is important to extend this as high as possible. Entering the chest at the membranous part of the diaphragm where the pericardium rests is annoying, even though anesthesia is under positive pressure, and such an opening, if made, must ultimately be closed. A self retaining retractor is introduced, and the intestines are packed caudad. Gentle traction downward on the duodenum then exposes the structures in the hepatoduodenal ligament, and dissection is started here.

The common bile duct is first freed, clamped, divided and ligated. In order ultimately to attain adequate length of portal vein, it is necessary to dissect the bile duct and hepatic ducts away from the vein in the hepatic hilum, and this is a good time to accomplish the dissection. The hepatic artery is now stripped, clamped, divided and ligated, freeing it and the gastroduodenal branch from the portal vein throughout its length. The remaining areolar tissue about the portal vein is cleared away to gain length on this vein. This is one of the most important factors in determining the ease of cannulation of this vein later. Therefore, it is our practice to free its branches in the hepatic hilum so they may ultimately be individually clamped and the vein divided flush with its bifurcation. We also dissect clean the other end of the portal back to the mesenteric-splenic junction. A braided silk ligature is now laid about the portal vein and attention is directed to the inferior vena cava. This is dissected as recommended by Firor and Stinson, freeing all the vessel from the hepatic hilum to the right lumboadrenal vein. In some dogs the right adrenal is closely applied to the cava in this region and must be carefully dissected away to gain sufficient length. When this segment of cava is freed, a braided silk ligature is passed about it and left loose.

A short anterolateral intercostal incision is now made on the right in about the 7th interspace and a small rib spreader is introduced. The lung is displaced upward and the inferior vena cava is dissected free from the diaphragm to auricle. A loose braided silk ligature is laid about this vessel. In our more recent cases, at Dr. Alfred Blalock's suggestion, we have occluded the aorta just above the diaphragm before opening the vena cava to minimize engorgement of the abdominal viscera with the resulting interstitial plasma loss after obstruction of the portal vein. If this maneuver is anticipated, the aorta is blindly dissected out just above the diaphragm and a braided silk ligature passed around the vessel. Occlusion is now

accomplished by sliding a cystic duct clamp down on the two limbs of this piece of braided silk until pulsations disappear in the abdominal aorta, and then locking the clamp with only the ligature in its jaws. This, of course, employs the principle of the Shenstone lung tourniquet. Control of the blood supply to the liver has now

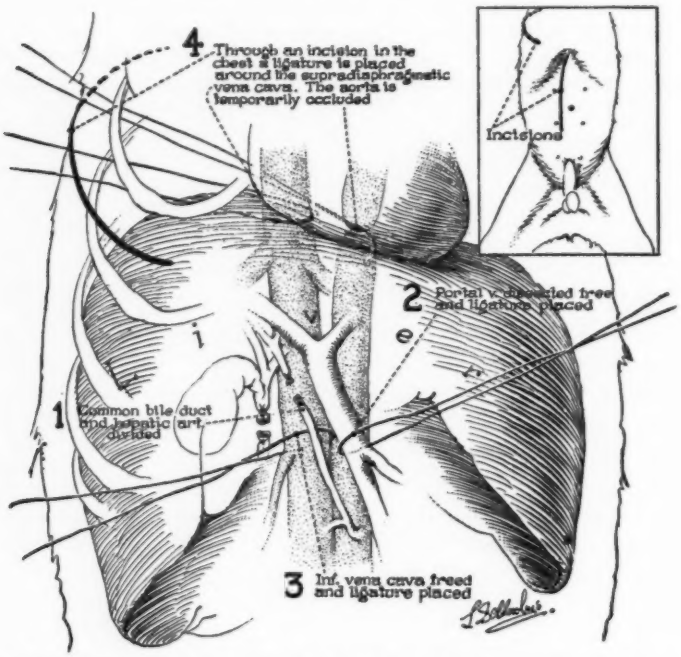

4
Through an incision in the chest a ligature is placed around the supradiaphragmatic vena cava. The aorta is temporarily occluded
Incisions
2
Portal v. dissected free and ligature placed
1
Common bile duct and hepatic art. divided
3
Inf. vena cava freed and ligature placed

FIG. 2. Initial steps in hepatectomy. Hepatic artery and common bile duct have been divided (1), portal vein and hilar vena cava have been dissected free and encircled by hemostatic ligatures (2, 3), and, through a small right anterolateral thoracotomy, supradiaphragmatic inferior cava and aorta have likewise been dissected free and encircled by hemostatic ligatures (4). The aorta has been temporarily occluded.

been obtained. The hepatic artery has been severed, and the portal vein is encircled by a controlling ligature, as is the vena cava below the hepatic hilum and above the diaphragm. In addition, the thoracic aorta has been temporarily occluded. Fig. 2 illustrates the procedure to this point. Attention is now turned once again to the abdomen.

A bulldog clamp is placed across the portal vein at the junction of splenic and

mesenteric veins. Small straight hemostats are placed on the branches of the portal vein at the hepatic hilum, and the vein is transected at this division point, thus obtaining the maximum length. The vena cava is then lifted up and occluded by a bulldog clamp at the point of ingress of the right lumboadrenal vein. The liver is then gently squeezed to milk out its contained blood, and, before it is released, a bulldog clamp is placed on the supradiaphragmatic cava just below the right auricle. This maneuver leaves the liver smaller and quite pale. It is now isolated from the circulation except for anastomoses with the phrenic veins in its bare area, and these can supply but little reverse circulation with the aorta obstructed.

The inferior vena cava is now grasped superficially with two mosquito hemostats about a centimeter below its disappearance into the liver. With a scalpel a longitudinal incision is made on its ventral surface for a distance of about 3 cm. This incision must not extend caudad closer than 2 cm. to the bulldog clamp on the cava, or there will not be enough room to ligate the vein about the cannula caudad to the extent of the venous slit. Length is easily gained on this incision by cutting boldly up into the hepatic substance at the hilum in a cephalad direction. The blood which escapes from the caval incision is that retained in the liver, and is not large in amount if the liver has been compressed as suggested prior to clamping the thoracic portion of the inferior vena cava. The edges of the caval incision are now grasped with mosquito clamps and held apart while the long end of the previously siliconed glass cannula is inserted into the vein and pushed cephalad until the short end falls into the cava through the posterior extent of the slit. Fig. 3 illustrates the procedure up to this step. The posterior end of the cannula is now slid caudad almost to the caval bulldog clamp, and the braided silk ligature is tied down about the cava over the posterior end of the cannula. Through the chest incision the supradiaphragmatic caval ligature is now tied down about the cephalad end of the cannula, which usually extends about 2 cm. above the diaphragm. The cannula is now flushed out by saline solution forcibly injected through a long needle attached to a plunger syringe and inserted through the side-arm of the cannula. This step is important, as several small clots are usually recovered, and the system is filled with fluid, displacing all air. The severed end of the portal vein is now held open by three mosquito hemostats, flushed out with saline, and slipped over the side-arm of the cannula with care not to twist the vein. A braided silk ligature is now tied about the portal vein on the side-arm of the cannula, and the circulation may now be reestablished. The vessels are opened in the following order to minimize visceral engorgement: supradiaphragmatic cava, portal vein, abdominal cava, aorta. Blood can now be seen streaming through the exposed parts of the cannula. It is desirable to have the animal awake as soon as possible after operation, and it has been found that this is best accomplished by discontinuing the ether at this point, and supplying only air under positive pressure until the chest is closed. If all goes smoothly, the circulation is interrupted about 8 minutes for cannulation, and peristalsis, defecation, and micturition occur after operation.

The animal is physiologically hepatectomized from the time of exclusion of the liver from the circulation, but the minimal circulation between the inferior phrenic

vessels and the bare area of the liver should be eliminated, and the huge, infarcted organ removed from the peritoneal cavity to eliminate imponderable factors from evaluation of postoperative studies. If the liver is removed now without ligating the cava below the diaphragm in the position originally employed by Firor and

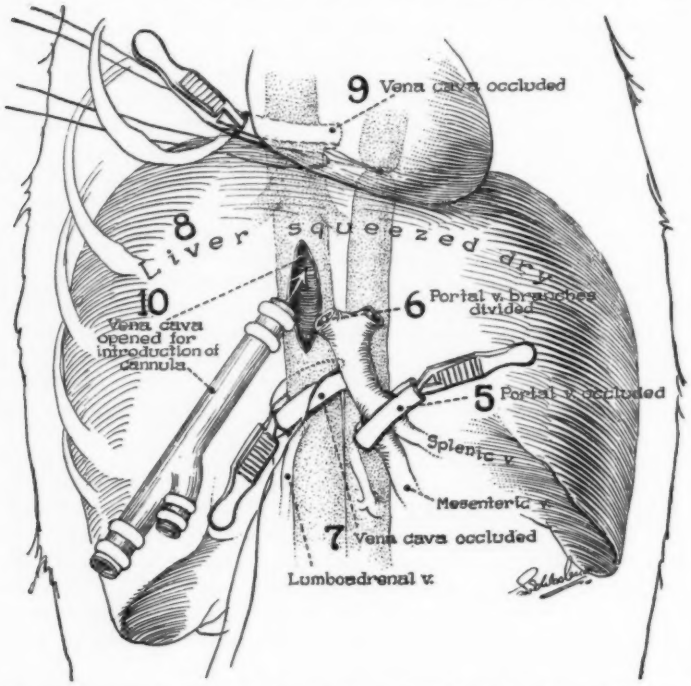


FIG. 3. Intermediate steps in hepatectomy. Portal vein has been divided at its hilar bifurcation (6) following its occlusion by a bulldog clamp (5). Vena cava has been occluded at entrance of right lumboadrenal vein (7) and blood has been squeezed out of the liver (8), following which the supradiaphragmatic vena cava has been occluded (9). Cannulation of the open vena cava is being performed (10).

Stinson, blood from the phrenic veins will enter the cava caudad to the ligature in the mediastinum, flow back around the cannula, and appear in the abdomen as a slow persistent, distressing ooze. Therefore a subphrenic ligature is employed as follows: the liver is drawn to the right and the gastrohepatic ligament is divided up to the coronary ligament which is divided to the cava. The liver is now displaced to the left and the peritoneal reflection over the cava is opened, the incision being carried as on the other side to the coronary ligament and then to the cava. At this

point the hepatic veins will be seen entering the cava. The subphrenic cava is bluntly dissected from its bed and a braided silk ligature passed behind it and tied down on the cannula. The liver can now be removed in one or more pieces by cutting

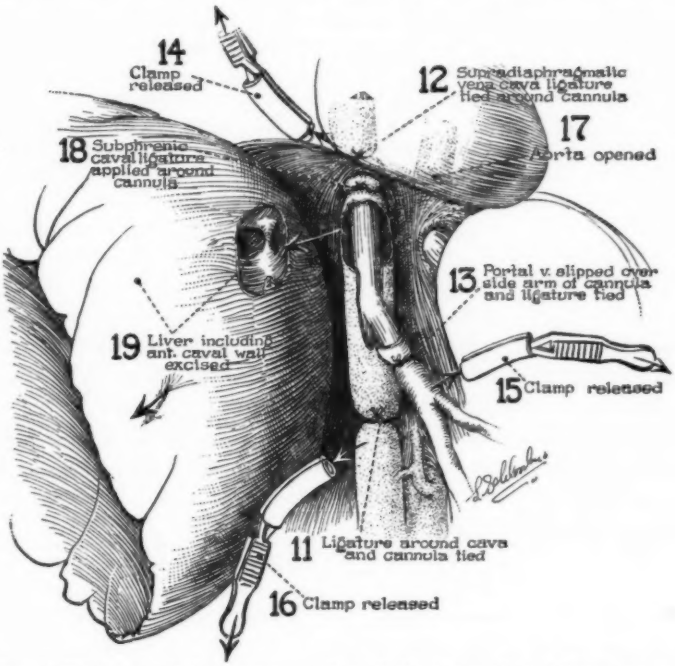

14
Clamp released
12 Supradiaphragmatic vena cava ligature tied around cannula
17
Aorta opened
18 Subphrenic caval ligature applied around cannula
13 Portal v. slipped over side arm of cannula and ligature tied
19 Liver including ant. caval wall excised
15 Clamp released
11 Ligature around cava and cannula tied
16 Clamp released

FIG. 4. Final steps in hepatectomy. Cannula has been slipped into vena cava which has been ligated about it above the right lumboadrenal vein and above the diaphragm (11, 12). Portal vein has been attached to cannula side-arm (13), and circulation reestablished by releasing occluding devices on supradiaphragmatic vena cava, portal vein, abdominal vena cava, and aorta in that order (14, 15, 16, 17). The infradiaphragmatic vena cava has been dissected free and ligated about the cannula (18), and the liver has been excised with the portion of ventral caval wall which receives the hepatic veins (19).

transversely across the hepatic veins and cava down to cannula, and carrying the ends of this incision caudad on either side of the liver through the caval walls until they meet the hilar slit in the cava through which the cannula passes. This frees the liver with the small segment of anterior caval wall to which it is intimately attached, and through which the hepatic veins enter. Fig. 4 shows these final steps in the procedure. Due to the marked post-hepatectomy bleeding tendency which

many of these animals display, it is essential to inspect carefully the operative area and ligate all bleeders, however small.

The lung is inflated and the ribs are approximated with braided silk about a small catheter. Muscles and skin of the chest incision are closed with continuous silk. Any residual air in the chest is aspirated through the catheter, which is then withdrawn. The abdominal incision is closed with a continuous suture of braided silk through all layers except the skin, which is closed with a continuous suture of fine silk. Recently a transfusion of 300 cc. citrated blood has been given at the conclusion of the operation, and the subsequent arterial pressure has been measured with a mercury manometer. The mean pressure has been 95 to 115 mm. of mercury, within normal range. The animal is extubated as soon after chest closure as the respirations are spontaneous.

RESULTS

In the process of developing this technique, we have extirpated the livers of ten animals who survived from 2 to 17½ hours after the liver was excluded from the circulation. Our observations of the clinical course following hepatectomy generally corroborate those of Mann and of Firor and Stinson. We administered glucose postoperatively, so that only the terminal phenomena of twitching, coma, vomiting, Cheyne-Stokes respiration and death were observed. However, only three of our animals survived intermediate hazards, notably those of hemorrhage, to develop this full picture.

Survival

Animals 1 to 5 were operated upon without aortic occlusion or postoperative blood transfusion and received intermittent injections of glucose after operation. The average survival of this group was 4½ hours. In animal 6, the aorta was temporarily occluded during the operation, and injections of glucose were given postoperatively. This animal lived 7 hours. Animals 7 to 10 each received intravenous infusions of a mixture of 5% glucose and 0.85% sodium chloride solution during and after the operation. The aorta was temporarily occluded, and a postoperative transfusion was administered. Their average survival was 9½ hours.

Cause of death

Post mortem examinations were made in each case. Death in the first two animals occurred after 2 and 3 hours respectively and was

due to intraperitoneal hemorrhage consequent upon failure to ligate the vena cava below the diaphragm. This step was thereafter incorporated into the technique. The third animal died after seven hours with the syndrome of coma, Cheyne-Stokes respiration, and terminal vomiting which, for want of a better tem, we shall call "liver death". The fourth animal died after 5 hours from an unrecognized pneumothorax with cerebral anoxia. The fifth animal died after 5½ hours with a general bleeding tendency. There were hemothorax and hemoperitoneum, despite dry operative fields at the occlusion of the procedure. Blood withdrawn from the heart after death was incoagulable. The sixth and seventh animals likewise slowly bled to death into chest and abdomen despite dry fields after operation. The *post mortem* blood of animal 6 formed a poor clot after 10 minutes, while that of animal 7 was incoagulable. Animal 8 was accidently killed by paradoxical air embolism to the coronaries following femoral puncture 5¾ hours after hepatectomy. This animal's blood was coagulable. Animals 9 and 10 lived 10½ hours and 17½ hours respectively and died "liver deaths". In each case, the blood was coagulable *post mortem*. These observations are summarized in the accompanying table.

DISCUSSION

The technique of hepatectomy which has been described has several advantages when compared with the method of Firor and Stinson (3) of which it is a modification. First, the temporary occlusion of the cava *above* the diaphragm before cannulation eliminates the danger of air embolism. Cannulation can be accomplished smoothly, in a relatively dry field, without haste or blood loss. There is no need to occlude the cannulas with stoppers, nor to place a finger on the aperture of the side arm, nor to cannulate the portal vein during retrograde bleeding from the side arm. Furthermore, the cannula can be flushed free of clots after it is tied into the cava, assuring its patency. Although small clots were always washed out when the cannula was flushed, not once was a cannula obstructed by clot thereafter.

Second, the dissection of the subphrenic cava is done in an almost dry field and at leisure, after the portacaval circulation has been reestablished. Our early attempts to perform this dissection without temporary occlusion of the supradiaphragmatic cava were attended by

considerable bleeding which partly obscured the field, and made it difficult to place the caval ligature properly.

Third, thoracotomy permits easy access to the aorta above the liver and allows temporary occlusion of this vessel before the portal vein is obstructed or the cava opened. This eliminates venous engorgement of the abdominal viscera and posterior parietes with the attendant transudation of plasma. We have not observed the paraplegia which often follows occlusion of the thoracic aorta in dogs. This may be due

TABLE 1

ANIMAL	AORTIC OCCLUSION	POST-OPERATIVE TRANS-FUSION	SURVIVAL TIME	CAUSE OF DEATH	COAGULABILITY OF BLOOD AFTER DEATH
			Hours		
1	No	No	3	No subphrenic caval ligation	Coagulable
2	No	No	2	No subphrenic caval ligation	Coagulable
3	No	No	7	"Liver Death"	Coagulable
4	No	No	5	Pneumothorax with cerebral anoxia	Coagulable
5	No	No	5½	Bleeding due to incoagulable blood	Incoagulable
6	Yes	No	7	Bleeding due to incoagulable blood	Delayed and imperfect clotting
7	Yes	Yes	4	Bleeding due to incoagulable blood	Incoagulable
8	Yes	Yes	5¾	Paradoxical air embolism after femoral puncture	Coagulable
9	Yes	Yes	10½	"Liver Death"	Coagulable
10	Yes	Yes	17½	"Liver Death"	Coagulable

to the brevity of obstruction or to the fact that the point of occlusion is so low. The presence of paraplegia, however, would not necessarily affect the value of the preparation for metabolic study.

Finally, it is possible to obtain a high percentage of animals which survive the operative procedure. In our experience, ten of eleven animals were successfully hepatectomized. The only animal in which we were unable to complete the procedure was one in which the junction of the splenic and mesenteric veins was separated from the division of the portal vein into its major branches by less than 1 cm. This al-

lowed too short a length of portal vein to permit cannulation, and the procedure could not be completed. This anatomical situation would probably have precluded successful hepatectomy by any of the previously described methods. The low operative mortality rate even during the development of this technical procedure may well be due to the elimination of air embolism and to the complete occlusion of the hepatic blood supply prior to hepatectomy.

Two minor disadvantages to the technique should be mentioned. First, unlike the method of Firor and Stinson, two incisions are required. The second incision, a thoracotomy, is small, rapidly made, and easily closed. Second, because of the presence of the thoracotomy, positive pressure anesthesia is needed. This is readily administered by using an endotracheal catheter with an inflatable pneumatic cuff, and an intermittent insufflator (7).

In hepatectomized dogs, a gradual fall in the concentration of fibrinogen in plasma has been described, suggesting that the liver may be the site of origin of this protein (8). Ordinarily, the decrease in the concentration of fibrinogen during the period of observation is moderate. Jones and Smith (9) found that the blood fibrinogen level fell 20 to 50% in 12 to 20 hours after a three-stage hepatectomy. We assayed the concentration of fibrinogen in the plasma in only one animal, No. 3. In this dog, the concentration of fibrinogen, determined by Quick's method (10), was 208 mg. per 100 ml. before hepatectomy, and 185 mg. per 100 ml. 7 hours after hepatectomy, values which do not differ significantly.

In three of the ten hepatectomized dogs, however, the blood either became incoagulable or clotted imperfectly and after some delay. In these three dogs, oozing hemorrhages occurred from the operative fields, so that death appeared to be the result of blood loss. These hemorrhages occurred despite the fact that careful attention was devoted to obtaining a dry field before closure of the incisions. Typically, large hematomas, involving all layers of the wounds, slowly developed in the postoperative period, and at necropsy, hemothorax and hemoperitoneum of major proportions were present. *Post mortem*, the hematocrit of these animals was as low as 11%.

The cause of the clotting defect in these animals was not elucidated. Probably the impaired coagulation was the result of a decrease in the

concentration of fibrinogen in the plasma. The fact that animals regularly undergo other complicated and prolonged procedures in our laboratories without developing such a clotting defect strongly suggests that this defect is the specific result of hepatectomy. None the less, in the animals which survived $10\frac{1}{2}$ and $17\frac{1}{2}$ hours, venous blood drawn *post mortem* clotted promptly. For this reason, it seems unlikely that hepatectomy alone can explain the impairment of coagulation in these animals.

The cause of the decrease in the concentration of fibrinogen in the plasma of hepatectomized animals is not understood. It has long been known that plasma contains a proteolytic enzyme system capable of digesting fibrinogen and fibrin. Nolf (11) showed that the fibrinolytic activity of plasma was activated by the intravenous injection of Witte peptone in hepatectomized, but not in normal dogs. Moreover, in human subjects, abnormally rapid fibrinolysis occurred in clotted whole blood (12) or plasma (13) of patients with hepatic diseases. Furthermore, rapid fibrinolysis was observed after sudden or violent death (14), in patients in severe shock (15), and in patients pre and postoperatively (16, 17). For these reasons, it seemed possible that the decrease in concentration of fibrinogen in the blood of hepatectomized dogs might be the result of the plasma's fibrinolytic activity. Only incomplete studies of this phenomenon were possible in the animals described in this paper. The oxalated plasma of dogs before and after hepatectomy was added to a solution of bovine fibrinogen, and the mixture was clotted with bovine thrombin. No evidence of significantly increased fibrinolytic activity was obtained in four animals studied with this technique. In five animals, the euglobulin fraction containing the bulk of the fibrinolytic activity was separated by acidification of diluted plasma or serum (18). The spontaneous proteolytic activity of this fraction, using casein as a substrate, rose slightly after hepatectomy. However, the degree of proteolytic activity seemed inadequate to explain the decrease in plasma fibrinogen after hepatectomy.

A second possible mechanism for the decrease in the concentration of fibrinogen in plasma is the utilization of this protein in intravascular clotting. The blood of dogs can be completely defibrinated *in vivo* by the intravenous injection of thromboplastic material (19). Perhaps sufficient thromboplastic material gains access to the circulating blood

in the hepatectomized dog to cause intravascular coagulation. In the absence of the liver, a decrease in plasma fibrinogen concentration might then result. It seems evident that the cause of the decrease in fibrinogen in hepatectomized dogs is worthy of further study.

SUMMARY

A modification of the one-stage hepatectomy described by Firor and Stinson is reported. In this procedure, the dangers of air embolism are minimized by temporarily occluding the inferior vena cava above the diaphragm through a thoracotomy wound. Ten of eleven dogs were successfully hepatectomized by this method, and two survived more than 10 hours. Three of the ten hepatectomized dogs developed gross defects in their blood clotting mechanism. This has not as yet been explained.

BIBLIOGRAPHY

1. Mann, F. C., Studies in the Physiology of the Liver. I. Technique and General Effects of Removal, Am. J. Med. Sci. **161:** 37 (1921)
2. Mann, F. C., The Effects of Complete and of Partial Removal of the Liver, Medicine **6:** 419 (1927)
3. Firor, W. M., and Stinson, E., Jr., Total Extirpation of the Dog's Liver in One Stage, Bull. Johns Hopkins Hosp. **44:** 138 (1929)
4. Firor, W. M., Personal Communication
5. Tarver, H., and Reinhardt, W. O., Methionine Labeled with Radioactive Sulfur as an Indicator of Protein Formation in the Hepatectomized Dog, J. Biol. Chem. **167:** 395 (1947)
6. Jaques, L. B., Fidlar, E., Feldsted, E. T., and MacDonald, A. G., Silicones and Blood Coagulation, Canad. Med. Assoc. J. **55:** 26 (1946)
7. Hanlon, C. R., Johns, T. N. P., and Thomas, V., An Apparatus for Anesthesia in Experimental Thoracic Surgery, J. Thorac. Surg. **19:** 887 (1950)
8. Madden, S. C., and Whipple, G. H., Plasma Proteins: Source, Production and Utilization. Physiol. Rev. **20:** 194 (1940)
9. Jones, T. B., and Smith, H. P., The Blood Fibrinogen Level in Hepatectomized Dogs and an Outline of a Method for the Quantitative Determination of Fibrinogen, Am. J. Physiol. **94:** 144 (1930)
10. Quick, A. J., The Hemorrhagic Diseases and the Physiology of Hemostasis, C. C. Thomas, Springfield, 1942.
11. Nolf, P., Des Modifications de la coagulation du sang chez le chien apres extirpation du foie. Arch. intern. physiol. **3:** 1 (1905)
12. Goodpasture, E. W., Fibrinolysis in Chronic Hepatic Insufficiency, Bull. Johns Hopkins Hosp. **25:** 330 (1914)

13. RATNOFF, O. D., Studies on a Proteolytic Enzyme in Human Plasma IV. The Rate of Lysis of Plasma Clots in Normal and Diseased Individuals. Bull. Johns Hopkins Hosp., **84:** 29 (1949)
14. YUDIN, S. S., Transfusion of Stored Cadaver Blood, Practical Considerations: The First Thousand Cases, Lancet **2:** 361 (1937)
15. TAGNON, H. J., LEVENSON, S. M., DAVIDSON, C. S. AND TAYLOR, F. H. L., The Occurrence of Fibrinolysis in Shock. Amer. J. Med. Sci. **211:** 88 (1946)
16. MACFARLANE, R. G., Fibrinolysis Following Operation. Lancet **1:** 10 (1937)
17. MACFARLANE, R. G. AND BIGGS, R., Observations on Fibrinolysis, Lancet **2:** 862 (1946)
18. RATNOFF, O. D., Studies on a Proteolytic Enzyme in Human Plasma. I. The Probable Identity of the Enzymes Activated by Chloroform and by Filtrates of Cultures of Beta Hemolytic Streptococci, J. Exper. Med. **87:** 199 (1948)
19. RATNOFF, O. D. AND CONLEY, C. L., Studies on Afibrinogenemia. II. The Defibrinating Effect on Dog Blood of Intravenous Injection of Thromboplastic Material—to be published.

"GIANT MOLECULES" AND CHOLESTEROL IN RELATION TO ATHEROSCLEROSIS*

ANCEL KEYS

Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minn.

The recent reports by Gofman et al. (1) on "giant molecules" in serum have aroused much interest. In the ultracentrifuge there is separation by flotation of substances containing varying proportions of lipoids, cholesterol and proteins. These are obviously important for the transport of lipid and lipid-soluble materials in the blood. At least some of these substances fall into fairly discrete classes of flotation characteristics and composition and special interest attaches to those which Gofman and his associates indicate as having possible diagnostic, prognostic and even etiologic significance in regard to the development of atherosclerosis. The emphasis has been on the particles which are characterized in the ultracentrifuge by -10 to -20 Svedberg sedimentation units. Gofman et al. refer to them as "S_f 10–20 molecules;" it is appropriate and convenient to call them the "G substances."

Several features in the reports by the Berkeley group have been confirmed by Lewis and Page (2) and by ourselves: 1) refractive index gradient patterns photographed during ultracentrifugation disclose the existence of differential flotation of a series of lipid-bearing substances of large particle size, 2) there are large differences between individuals in the patterns thus obtained but given individuals tend to have characteristic patterns, 3) serum which is hypercholesterolemic or is from persons with evidence of atherosclerosis tends to show relatively high concentrations of G substances.

The arguments for the importance of the G particles in regard to atherosclerosis are, briefly: 1) the concentration of G in patients who have had a myocardial infarction tends to be higher than in persons who are clinically normal, 2) patients with diseases frequently associated with early and severe atherosclerosis—diabetes mellitus, nephrosis, hypothyroidism—tend to have high concentrations of G in the

* This paper represents part of a De Lamar Lecture presented on February 6, 1951, at the School of Hygiene and Preventive Medicine of the Johns Hopkins University.

serum, 3) in rabbits fed large amounts of cholesterol there is a high correlation between the concentration of G in the serum and the degree of atherosclerosis as graded by the pathologist.

The same arguments, however, apply to the concentration of total cholesterol in the serum so a major question is as to how the concentrations of G and of cholesterol compare in regard to these problems. The California group suggest that the concentration of G is of much greater significance than that of cholesterol in regard to atherosclerosis and, further, state that "for any particular patient the analytical serum cholesterol is of no value in predicting the concentration of S_f 10–20 molecules" (1B, p. 174). The actual relationships, however, are impossible to discern without appropriate analyses. Though such analyses

TABLE 1

Correlation between the serum concentrations of G and of total cholesterol in men studied by Gofman, et al. (1950 B)

r is the product-moment coefficient of correlation: "95% limits" gives the range within which we should expect to find the value of r in 95 per cent of similar samples of data.

NO.	MEN	r	95% LIMITS
89	Normal	+0.56	0.39 to 0.70
98	Infarcts	+0.48	0.31 to 0.63
187	Total	+0.56	0.45 to 0.66

are absent from the publications on G, some of them can readily be made from the raw data in the published scatter diagrams.

CORRELATION BETWEEN G AND CHOLESTEROL

From Figures 14 and 15 of the main paper by Gofman et al. (1B, p. 171), it is possible to tabulate the concentrations of G and of total cholesterol for each of 89 clinically normal men and 98 men who had had myocardial infarctions. The correlations between these two characteristics for each of the groups and for all 187 men are summarized in Table 1.

The correlations given in Table 1 are highly significant but imperfect. However, these computations provide only minimal estimates of the actual degree of relationship between G and total cholesterol concentrations because of the fallibility of the methods used to obtain

the raw data. The analytical error in the measurement of total cholesterol is not small and that for G is very substantial. The limit of resolution for G was stated by Gofman et al. (op. cit.) to be 5 mg. per 100 ml., but a more recent personal communication from Dr. Gofman indicates that the precision is considerably less than this. From our own experience and that of others with whom we have compared notes the uncertainty of a single G measurement seems to be of the order of ± 10 mg. Since 42 per cent of the 187 readings at hand for correlation analysis are only 15 mg. of G or less, the effect of a random uncertainty of this magnitude is large. In the absence of an acceptable reliability analysis no precise estimate can be made of the true correlation which would be found if infallible methods were available. It is evident, however, that the true relationship between G and total cholesterol concentrations must be closer than indicated by the crude value of $r = +0.56$.

DISCRIMINATION BETWEEN NORMALS AND INFARCTS

The California workers have properly emphasized the importance of differences between the serum concentrations in clinically normal men and in those men (with infarcts) who must be presumed to have developed an important degree of atherosclerosis. The published analysis, however, fails to indicate the confidence which may be attached to the discrimination in terms of levels of G and does not examine at all the question of discrimination in terms of cholesterol levels. The bar diagrams (Figs. 10, 11, 12, op. cit.) summarizing the G data depict arithmetic averages which are of dubious value where the frequency distribution is, as is the case with G, skewed in extreme degree; for the same reason, simple standard deviations or errors for G would be misleading. We can, however, examine such data with profit by comparing the proportions of normal men and of men with infarcts found at or above given concentration levels. We have attempted to make such analyses in several ways.

In the first place, we arranged the combined data (extracted from Figs. 14 and 15, op. cit.) from both infarcts and normals in an array from lowest to highest values of G and, independently, from lowest to highest values of total cholesterol. These arrays were then divided into ten successive groups of equal size (deciles) and the numbers of

normals and of infarcts in each decile were ascertained. When these numbers are expressed as percentage of the total normals and of the total infarcts and these percentages are cumulated by successive deciles, the results indicate the capacities of measurements of G and of total cholesterol to discriminate between these "normal" men and these men with infarcts. The analysis indicates that cholesterol measurements tend to separate the two categories more sharply than do G measurements at deciles 4, 5, 6, 7, and 8, and that the two criteria do not differ in discrimination at deciles 1, 2, 3, and 9.

A somewhat analogous procedure is to select convenient levels for G and for total cholesterol concentrations and to ascertain from the data the percentages, of normals and of infarcts, separated by these levels. The results are summarized in Figure 1. The discriminating power for any level of either G or cholesterol in Figure 1 is simply the ratio of the "Infarcts" ordinate to that for "Normals" at the same abscissa.

These analyses, together with further appropriate statistical treatment, indicate that both G and total cholesterol do, in fact, discriminate in this material between normals and infarcts with statistical significance, but that the discriminating power of total cholesterol may be greater than that of G.

Copies of these analyses and the statistical treatment were sent to Dr. John Gofman for his information and comment. In his reply he stated: "You used our table of normals S_f 10–20 vs. cholesterol and compared it with our infarcts S_f 10–20 vs. cholesterol. This is completely incorrect, since the infarct data are almost wholly for males 40–70 years of age whereas the normals are predominately in the 20–40 bracket, though several are over 40."

This is the first intimation that the data in Figs. 14 and 15 (op. cit.) are not directly comparable but it remains to be seen what may be the effect of this difference in the ages of the two groups. The curves for "infarcts" in Fig. 1 here are not affected but the curves for "normals" need adjustment in so far as "normals" have an age trend in values of G and of total cholesterol in the serum. An age trend for serum cholesterol concentration in normal men is well established; there is a progressive rise with age until the fifties and a tendency to decline after age 60 (3). There is a similar age trend for the G con-

centration in normal men—a progressive rise with age until the fifties and a tendency to decline after age 60 (1B, Fig. 11).

It is clear, therefore, that an older group of normal men would yield "normals" curves for our Fig. 1 which would be closer to the "infarcts" curves for both G and cholesterol. Figure 1 *overestimates*

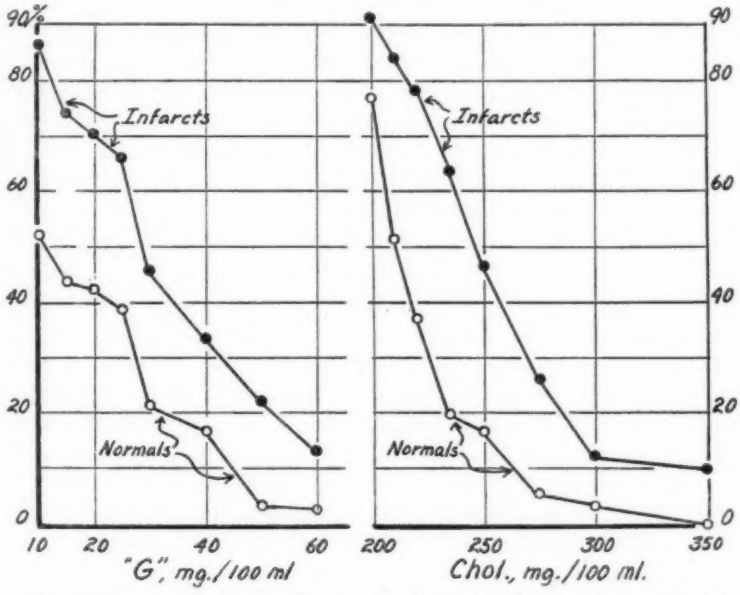


FIG. 1. Percentages of normal men and of men who have had myocardial infarctions who are found to have blood serum concentrations equal to or higher than the values indicated on the abscissae. The same population of 89 "normals" and 98 "infarcts" is depicted in regard to the concentrations of G and of total cholesterol. Calculated from raw data published by Gofman et al. (p. 171, 1950 B).

the capacity of measurements of both G and of total cholesterol to discriminate between normals and infarcts. However, if the normal age trend were the same for G and cholesterol concentrations, the relative discriminating power of the two criteria compared with each other would be unaffected. But in the absence of detailed data on the age composition of the normal and infarct groups in Fig. 1, and of a proper analysis of the normal age trend for G, precise adjustment is

impossible. We can only conclude that in a mixed population of younger normals and older infarcts, cholesterol measurements tend to excel G measurements in discriminating between normals and infarcts. Further, neither measurement affords sharp discrimination except at very high values of G and of cholesterol which include only some 10 per cent of the cases of infarction.

One final point on the data summarized in Fig. 1 may be mentioned briefly. In view of the fact that G and total cholesterol are only imperfectly correlated with each other but both are correlated, also imperfectly, with the presence of infarction, their combined effect should be explored. Several methods of analysis have been applied for this purpose and all give essentially the same result. It is easy to find combinations of G and cholesterol levels which have considerably more discrimination between infarcts and normals than any level of G alone (e.g. G = 20 and chol. = 200 or 220, G = 10 and chol. = 200 or 220, etc.). But systematic trials failed to find any combination of G and cholesterol levels as good as the best level of cholesterol alone (chol. = 235). In other words, for discriminating between younger normals and older infarcts, the measurement of G seems to make no independent contribution beyond that obtained by cholesterol measurement alone.

The foregoing analysis does not show precisely how well the measurement of G discriminates between men of *equal* ages who are, respectively, clinically normal or have infarcts. Such an analysis is not provided by Gofman et al. but can be made from the raw data in their Figs. 4 and 5 (1B, pp. 166 and 168). The essentials for such an analysis are summarized here in Table 2.

An index of the power of the G measurement to concentrate infarcts can be provided directly from Table 1 by computing the ratios of infarcts to normals at each level. For example, in a population containing equal numbers of infarcts and normals, above 20 mg. of G per 100 ml. of serum there will be roughly twice as many infarcts as normals while above 40 mg. the ratio will be more nearly 3. While the power of G to "find" infarcts is not very impressive, it should be observed that values of G below 5 mg. may have more significance in the converse direction; at such very low levels there will be, relatively, something like 5 to 8 times as many normals as infarcts.

Table 2 indicates the nature of the age trend among normal men but it will be observed that the discrimination of G between normals and infarcts is much the same at the two age ranges considered.

These results may be compared with the findings with cholesterol reported by Gertler, Garn and Lerman (4A) in the same issue of

TABLE 2

Discrimination between normal men and men with infarcts by means of measurements of G (mg. per 100 ml. of serum)

Computed from data from Gofman et al (1B, Fig. 4, p. 166, and Fig. 5, p. 168). N = "normals," I = "infarcts."

AGE	TYPE MEN	NO. MEN	% MEN WITH G ABOVE		
			5	20	40
years					
31–50	N	483	60.5	33.8	11.4
	I	72	95.8	73.6	34.7
51–60	N	99	66.7	34.4	16.2
	I	75	93.3	68.0	38.7

TABLE 3

Discrimination between normal men and men with infarcts by means of total cholesterol measurements (mg. per 100 ml. of serum)

Computed from data from Gertler, Garn and Lerman (4, Table 1, p. 207). G_I/G_N gives the ratios obtained from Table 2 by dividing line 2 by line 3; these are the comparable values for discrimination by means of G measurements.

	CHOLESTEROL ABOVE		
	209	239	299
% Normals	60.3	35.6	5.5
% Infarcts	88.7	73.2	41.2
I/N	1.47	2.06	7.49
G_I/G_N	1.58	2.18	3.04

Circulation in which the G data appeared. The Boston study covered 97 men who had myocardial infarctions and 146 "healthy" men of the same age. The ages of these men (4B) make it proper to compare them with the 31–50 year category in Table 2. It is fortunate that it is possible to find in the Boston data cholesterol levels which divide normal men at much the same percentage as do the G data in Table 2. The results of the computations are given in Table 3.

Obviously, this comparison is in essential harmony with the previous analyses in this paper. All of the evidence indicates that to "find" infarcts the measurement of the concentration of G in the serum is certainly not superior to but, if anything, is inferior to the measurement of total cholesterol, particularly at very high concentrations of these substances which occur in less than 5 or 10 per cent of normals. In so far as the argument that G measurements have unique virtue in "finding" atherosclerosis depends on its performance in discriminating between infarcts and normals, the argument must be denied.

TABLE 4

Correlation between the degree of atherosclerosis in cholesterol-fed rabbits and the serum concentrations of G and of total cholesterol

Calculated from data from Gofman et al (1950 B) and from Firstbrook (1950). The "95% limit" gives the range within which we should expect to find the value of the correlation coefficient, r, in 95 per cent of similar samples of data.

ITEM	N	r	95% LIMIT
G.	19	0.77	.48 to .91
Tot. Chol.	14	0.85	.57 to .95

RABBIT EXPERIMENTS

The published evidence for the conclusion that the concentration of G is directly related to the extent of the atherosclerotic process is contained in the scatter diagram labelled Fig. 3 in reference 1B (p. 167). This shows the degree of atherosclerosis, graded 0 to +4, in 19 rabbits fed cholesterol, and the corresponding concentrations of G in the serum. At the same time (August, 1950) that these data appeared, a close parallel was published by Firstbrook (5) who studied total cholesterol in the serum and the degree of atherosclerosis, graded on the same scale of 0 to +4, in 14 rabbits fed cholesterol. The two sets of data are summarized in Table 4.

The correlations between degree of atherosclerosis and G, on the one hand, and total cholesterol, on the other, are both very high. Although the coefficient of correlation is higher in Firstbrook's series, the two values are not significantly different in these small series. If there is a difference between these two measures of the tendency of rabbits to develop atherosclerosis on a high cholesterol intake, considerably longer parallel series would be required to demonstrate it.

COMMENT

The discussion here has been confined primarily to elementary questions about the relationship between G and total cholesterol concentrations in the serum and the frequency incidence of different concentrations of these substances in clinically normal men and in men who had had myocardial infarctions. From the analysis it is clear that if we merely wish to distinguish between "normals" and "infarcts" some success will be attained with either G or total cholesterol concentration measurements and that, if anything, total cholesterol may prove better than G for this purpose.

All of this is merely an indirect approach to the possibility of detecting or estimating the intensity of the development of atherosclerosis. It is true that there is a high degree of probability that men with infarcts have established an important degree of atherosclerosis but there is no evidence at all that the atherosclerotic process was active at the time the blood samples were drawn. Moreover, it is probable that some of the "normals" were actually in an active stage of developing atherosclerosis when the bloods were drawn. In other words, the mere presence or absence of clinical evidence of previous infarction is a questionable criterion for the presence or absence of the active process at the moment in any given individual.

The evidence on rabbits fed cholesterol is of a much more direct nature but the limitations, again, must be observed. We are entitled to conclude only that, in the rabbit, when extraordinarily high levels of cholesterol in the blood are produced and maintained by cholesterol feeding, then there is a good correlation between the extent of aortic atherosclerosis and the concentration of G in the serum. There is not the slightest evidence that this correlation surpasses the correlation obtained with cholesterol measurements alone. Moreover, the relevance of this evidence from rabbits to actual atherosclerosis in man is by no means clear since no such levels of cholesterol in either diet or blood or of G in the serum are involved in man and even the distributions of the lesions is quite dissimilar.

The thesis that the concentration of G in the blood has any significance at all for human atherosclerosis requires many assumptions. But if those assumptions are made, then it appears that the concentrations of total cholesterol has at least as much significance as has G.

This is about all that can be said from evidence so far available. It is conceivable that the concentration of G will eventually prove to be a useful predictor of future coronary disease. It is equally conceivable that it will prove to be of less value than simple cholesterol.

In any case, the development of atherosclerosis clearly depends on factors in the arterial wall as well as the presence or concentration of particular substances in the blood. Moreover, the development of atherosclerosis is a gradual process extending over many months, years or decades in man. In so far as the blood concentration affects this process, it must be thought of as a summation extending over all this time. The significance of a blood measurement at any one moment is obviously limited unless there is proof that such a sample does accurately portray the whole past history.

SUMMARY

From an analysis of published data it appears that there is an important correlation between the concentrations in the blood serum of total cholesterol and of the "giant molecules" or aggregates (G) characterized by -10 to -20 Svedberg units in the ultracentrifuge. The correlation coefficient in both normal men and in men with myocardial infarctions is of the order of at least 0.5 to 0.6.

As a criterion to discriminate between clinically normal men and men with the important degree of atherosclerosis represented by myocardial infarction, the measurement of G is not superior to and may well be inferior to the measurement of total cholesterol in the serum.

Extremely low values of G in serum are rare in men with infarcts, hence the finding of such low values may be useful in estimating the likelihood of normality. On the other hand, very high values of total cholesterol in the serum tend to be more frequently related to atherosclerosis than are correspondingly high values of G.

There is no evidence that combining G and total cholesterol criteria will improve predictions of abnormality made from cholesterol measurements alone.

Data from cholesterol-feeding experiments with rabbits show the severity of atherosclerosis which results is highly correlated to about the same degree with both G and total cholesterol levels in the serum,

but that the effective serum levels are beyond the range usually seen in man.

At the present time, it is entirely unjustified to attribute to G measurements any special virtue beyond that for simple cholesterol measurements for the prediction of atherosclerosis or the estimation of the activity of the atherosclerotic process.

REFERENCES

1 A. Gofman, J. W., Lindgren, F., Elliott, H., Mantz, W., Hewitt, J., Strisower, B., and Herring, V., The role of lipids and lipoproteins in atherosclerosis. Science 1950, **111:** 166.

1 B. Gofman, J. W., Jones, H. B., Lindgren, F. T., Lyon, T. P., Elliott, H. A., and Strisower, B., Blood lipids and human atherosclerosis. Circulation 1950, **2:** 161.

2. Lewis, L. A., and Page, I. H., Ultracentrifuge and electrophoretic studies on serum lipoproteins in relationship to vascular disease (abstract). Circulation 1950, **2:** 466.

3 A. Keys, A., The physiology of the individual as an approach to a more quantitative biology of man. Federation Proc. 1949, **8:** 523.

3 B. Keys, A., Mickelsen, O., Miller, E. v.O., Hayes, E. R., and Todd, R. L., The concentration of cholesterol in the blood serum of normal man and its relation to age. J. Clin. Invest. 1950, **29:** 1347.

4 A. Gertler, M. M., Garn, S. M., and Lerman, J., The interrelationships of serum cholesterol, cholesterol esters and phospholipids in health and in coronary artery disease. Circulation 1950, **2:** 205.

4 B. Gertler, M. M., and Garn, S. M., Cholesterol, cholesterol esters and phospholipids in health and in coronary artery disease. Science 1950, **112:** 14.

5. Firstbrook, J. B., Effect of choline chloride on development of atherosclerosis in the rabbit. Proc. Soc. Exp. Biol. Med. 1950, **74:** 741.

MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

Monday, February 12, 1951

Dr. Fisher: We have a very interesting program this evening. It is a rather long one and I think we had better get started. The first paper is by Dr. R. T. Shackelford.

EXPERIENCES WITH PERITONEOSCOPY

In 1948 I collected the reports on peritoneoscopy in the literature, (Shackelford, R. T., Lewis System of Surgery, Vol. XI, Chap. 35), and found that in 7000 cases there had been ten deaths.

My own personal experience is limited to 229 peritoneoscopies performed upon 195 patients, some of the patients having undergone the procedure more than once in order to follow the progress of their disease.

Peritoneoscopy is always performed in an operating room and the patient spends the night after operation in the hospital.

Local anesthesia is used for adults, and sodium pentothal, ether, or local for children.

The report of my results will be based only upon the 97 cases whose complete records are available, and the other 98 cases will be excluded because of lack of data caused by discontinuity or loss of their files during my absence in the war.

The complications in this series are seen in Table I.

The diagnoses established in this series are listed in Table II.

In nine instances an incorrect diagnosis was made.

In the 97 cases a futile laparotomy was prevented in 27 instances of inoperability. Twenty-six patients were operated upon for what was shown to be either a benign lesion or a malignant lesion that was operable. In 30 cases the diagnosis of cirrhosis, hepatitis or tuberculous peritonitis was unequivocally established so that the patient could be appropriately handled with assurance.

From this experience one can conclude that peritoneoscopy is a useful and relatively innocuous procedure for a small group of carefully selected cases, and that it is most useful in differentiating diseases of the liver, determining the obscure cause of ascites and, differentiating female pelvic lesions in those cases in which a culdoscope is either unavailable or contraindicated.

Dr. Fisher: Dr. Shackelford's paper is open for discussion. Is there any discussion or are there any questions?

Dr. Ravitch: It is always an asset to a department or an institution to have someone who will take up a new instrument or diagnostic method and who will become accurate and skillful in its use and careful in its evaluation. We have seen

a beautiful demonstration of the sort of thing one can observe by this method of intraperitoneal study in Dr. Shackelford's presentation.

TABLE I

Total patients peritoneoscoped	97
Unsatisfactory peritoneoscopy (adhesions)	5
Satisfactory peritoneoscopy	92
With biopsy	31
Without biopsy	61
Complications:	
Perforated bowel	1
Postoperative hemorrhage	0
Postoperative pain	3
Postoperative emphysema	1
Deaths	0
Exacerbation of symptoms (fever)	2

TABLE II

Satisfactory peritoneoscopies	92
Correct diagnoses	83
Cirrhosis of liver	19
Combined cirrhosis and tuberculous peritonitis	1
Normal liver and peritoneum without metastases	16
Metastases, source invisible	13
Carcinoma of ovary, inoperable	7
Operable	2
Hepatitis	7
Tuberculous peritonitis	3
Ectopic pregnancy, present	2
Absent (1 positive Friedman's Test)	2
Myomatous uterus, post-menopause	1
Pre-menopause	1
Ovarian cyst	2
Primary hepatoma	2
Peptic ulcer	2
Carcinoma of gall bladder	1
Obstructive jaundice due to stones	1
Retroperitoneal tumor	1
Total	83
Incorrect diagnoses	9

Endoscopy probably provides the biggest thrill that we essentially visual and tactile physicians in surgery get. Indigocarmine spurting out of the ureteral orifice during endoscopy of the bladder is as pretty a sight as anyone can see. With the new instruments practically every bit of the surface of the bladder can be seen.

Nasopharyngoscopy is pretty satisfactory for exploration and the external auditory canal is entirely accessible. Bronchoscopy certainly reveals everything about the mucous surface of the trachea and bronchi, and esophagoscopy is highly reliable. With all of these organs with easily definable shape and contour, illumination and exploration are satisfactory. This is less true of the stomach in gastroscopy.

However when we come to the peritoneal cavity, we have an enormous surface, the actual surface of the parietal and visceral peritoneum amounting to many square meters, so disposed that little of it is accessible. This is the more so when one considers the relatively small field of vision which must definitely limit the utility of such an instrument as the peritoneoscope, attractive as the idea may be.

One would wish to raise a number of questions about experiences with peritoneoscopy. I think most of us have the feeling that the most brilliant application of such an instrument has been in the pelvis, using culdescopy, to which Dr. Shackelford referred, when one is anxious to differentiate between pelvic inflammatory disease and a tubal pregnancy,—and as the additional protection is the fact that within 24 hours one can obtain biological confirmation or refutation of one's diagnosis. That is not possible within the abdomen and in dealing with carcinoma one can tell nothing through the peritoneoscope about the preaortic glands, the condition of the kidneys or adrenals or many other structures. Within the abdomen I am sure that many times we may be saved an unnecessary exploration. For instance with a patient with fairly obvious carcinomatosis who can be sent home with a definitive diagnosis of hopeless carcinomatosis if tissue can be obtained for pathological study.

I wonder if Dr. Shackelford would be able to tell us from his figures, for instance in the 16 patients in whom the liver was found to be normal, what the ultimate course was? I wonder whether it was possible to tell in how many patients an operation was avoided that would otherwise have been performed, and on how many patients an operation was advised and usefully performed that would otherwise not have been done. A diagnostic method that has a proven fallibility in 9 or 10% of the cases subsequently explored is not highly dependable. Nevertheless there is a range of usefulness for the procedure and it may be of value, especially if carefully restricted to the indications mentioned by Dr. Shackelford. There is of course no possible question about the inadvisability of use of peritoneoscopy in any acute inflammatory condition of the abdomen.

Dr. Fisher: Are there any questions anyone else wants to ask? Dr. Shackelford, do you want to close?

Dr. Shackelford: I am delighted that Dr. Ravitch raised some of the questions that he did, which I think are very good questions. And let me take them up somewhat in reverse order, if you don't mind.

In the first place, there are contra-indications to peritoneoscopy. I was running over my time and didn't mention them. I do not think a patient should be peri-

toneoscoped who has an acute abdomen. Secondly, I do not think a patient should be peritoneoscoped who has had several previous abdominal operations. The reason for that is there are undoubtedly, or nearly always, adhesions secondary to those operations that will limit the usefulness of the peritoneoscope. And the third contra-indication, I think, is a patient with a distended abdomen, distended by gas, because a thinly distended bowel would appear to be more liable to perforation than one that is not.

Now, to return to a couple of Dr. Ravitch's specific questions. The sixteen cases that I referred to were taken because I had the case histories on those sixteen and no evidence of malignancy has been subsequently developed from these. In the other ninety-eight cases, I didn't know what the ultimate outcome of those patients was and therefore did not include them in this particular series.

It is perfectly true that metastases to the glands will not be seen, that is, to the deeper glands. Quite frequently you see metastases to the glands in the omentum and, of course, usually you see metastases to the liver.

In conclusion, I want to say that this procedure is not one to be used as a general excursion around the abdomen for sight-seeing purposes. Rather, it is one to be used to decide a particular differential point and particularly, I think, in connection with the liver, because the liver is sort of a blind spot in the abdomen as far as our being able to detect lesions within it. We have to depend on indirect evidence and here is a means of having direct evidence of what is taking place in the liver. So again I say that peritoneoscopy should be performed only on those cases in which you want to make a specific differential diagnosis and preferably a differential diagnosis that concerns the liver.

Dr. Fisher: The second paper is by Drs. J. D. Frank and Eduard Ascher. The paper is to be given by Dr. Frank.

CORRECTIVE EMOTIONAL EXPERIENCES IN GROUP PSYCHOTHERAPY

The goal of dynamically oriented psychotherapy is to bring about changes in a patient's attitudes which will enable him to function more satisfactorily and to utilize his assets more fully. Such attitudinal changes were observed in patients undergoing psychotherapy in groups after the occurrence of a crucial event which appeared to have had special significance to the patient. Several processes are recognized to favor the occurrence of such an event. They are (1) emotional support by other patients and the doctor, (2) stimulation of attitudes as afforded by the group setting and (3) opportunity for reality testing in the group which represents society in miniature. Support is often given by sympathetic appreciation of one's lot by others. Attitudes toward important persons are frequently stimulated by one or several group members who remind the patient of those persons, and reality testing can take place owing to the real life character of the group setting which brings distortions into the open and permits examination.

Two clinical examples of patients who had a corrective emotional experience in group therapy illustrate the nature of these therapeutic processes. The event itself consisted of reexperiencing an important recurrent, emotionally painful reaction in a setting that was similar to those which produced it outside the group, but leading to a different outcome. Persisting beneficial attitudinal changes seemed to follow the experience.

Dr. Fisher: This paper is open for discussion. Dr. Ascher, would you like to add anything?

Dr. Ascher: No, I have nothing to add.

Dr. Whitehorn: It is dangerous to call on me, because I feel inclined to make a speech too. The presentation of these two papers together at this same time provides an opportunity to make a comment that might be pertinent. You can see into the abdomen by the peritoneoscope. And by group therapy it is possible to introduce an observer into the setting of the group. That is why Dr. Frank is able to bring you such a detailed account. The observer was seeing what went on, watching the impressions made in the situation. The keenness of the reporting of the situation depends upon the possession of a psychotherascope. I think perhaps group psychotherapy has its greatest significance in bringing events of psychotherapy open to view and to the keen study of somebody (other than the doctor) who is watching what is going on all the time. This is the speech I was going to make. I would like to ask a question, too. It is of great importance to all kinds of psychotherapy.

It would appear here, and I would like to ask Dr. Frank or Dr. Ascher if they agree with this impression, that the success of these two incidents in group therapy seems to highlight the importance of keeping the therapeutic discussions focused on actual life situations of these people. What do you think?

Dr. Fisher: Are there any other questions or discussion? Just as in the group, everybody has a chance.

Dr. Frank: I would underline what Dr. Whitehorn has said. I have, little by little, realized how much more preoccupied I used to be with the content of what patients were saying, as if talking of such matters as infantile sexual experiences or childhood events were the important thing. However, in group therapy, one concentrates on emotional experience at the present time, and more and more my interest has been to conduct the groups in such a way that something emotional happens, and it is relatively unimportant what they happen to be talking about. When real emotions thus are aroused they bring to light what it is that is really bothering the patients.

Dr. Fisher: The last paper is by Drs. F. P. Chinard and L. B. Flexner. Dr. Chinard will present the paper.

CAPILLARY PERMEABILITY

The generally accepted version of the Starling hypothesis on the transcapillary movement of extracellular fluid has two major features. The first concerns net shifts of water from blood to interstitial spaces or in the reverse direction as determined by the hydrostatic pressures and protein concentrations on the two sides of the capillary walls. The second feature relates to the manner in which fluid passes from blood to interstitial spaces and returns in individual capillaries.

The current concept on the passage across the walls of individual capillaries posits passage in bulk by a process of filtration of protein free fluid at the arteriolar end of the capillary, circulation of this fluid in the interstitial spaces and return to the blood at the venular end by a process of diffusion. This concept contains an internal contradiction: substances of high molecular weight and low diffusion coefficients should accumulate in the interstitial spaces relative to substances with high diffusion coefficients; but experimentally such differing substances as inulin and thiocyanate are found to have approximately equal distribution volumes.

On the basis of earlier work with heavy water and radioactive sodium in whole animals, Flexner and his collaborators had suggested that movement across the capillary wall occurred by a process of diffusion in either direction over the whole length of the wall. Objections can be raised to this approach; it was decided therefore to study the mechanism of passage in individual organs of the dog.

"Instantaneous" injection was made into the main vessel supplying blood to an organ and samples of blood were obtained from the main vessel draining blood from that organ. The injection mass usually consisted of inulin and thiocyanate, whose diffusion coefficients have a 1:10 ratio, and the blue dye T-1824; this dye is assumed not to leave the blood stream in significant amount and could therefore be used as reference. Evidence of separation of inulin and thiocyanate was found in the limbs, head, liver, spleen, and kidneys. The data were interpreted as indicating that the substances studied left the blood stream by diffusion.

An alternative working hypothesis was presented. In this it is considered that movement of a substance may take place back and forth across the whole length of the capillary. Net movement may take place as the result of differences of the chemical potentials of a given substance on the two sides of the wall by a process of diffusion. This net movement is small compared to the rates of exchange across the capillary wall.

Dr. Fisher: This paper is now open for discussion. Dr. Flexner?

Dr. Flexner: Thanks, I have nothing to add.

Dr. Clark: I am delighted that Dr. Chinard has pointed out two absolutely distinct aspects of what he calls the Starling theory. I am, however, a bit provoked that he attaches Starling's name to the dynamic aspect. When we remember the period (1896) at which Starling proposed his theory I feel that we should grant to Starling the use of then ill defined terms such as *filtration* (I believe he

also used *transudation*) which carry certain implications of mechanism. I have always considered the essence of Starling's theory to be simply this: The excess solutes in plasma over that in interstitial fluid and the excess pressure in capillaries over that in interstitial fluid have opposite effects upon the properties of water; the excess solutes lower the vapor pressure, lower the escaping tendency, lower the chemical potential (use what terms you will) of the water; the excess pressure increases the escaping tendency of water. Were the opposing effects to balance, there would be no *net* movement of water at the equilibrium state.

Now the equilibrium state, in the case at hand, is an ideal reference state. It is exhibited in an osmometer and there the term osmotic pressure has meaning. The term never should be used for a non-equilibrium state. The standard of reference has served admirably. But strictly it is nothing more and cannot apply to the dynamic changes Dr. Chinard has been discussing.

Thus I have always considered the Starling theory to be concerned only with *net* changes relative to an equilibrium state of an ideal standard.

When we come to the finer dynamic aspects we encounter all sorts of complexities —"leakage" of protein, if you want to call it that, the return of protein *via* the lymphatics and the metabolism of proteins, innumerable variations of pressures, exchanges between plasma and red cells and plasma and interstitial fluid as the blood runs through a capillary, etc., etc.

Strangely enough, some people in dealing with capillaries speak of filtration, "flow" through pores, etc., while in more general treatments they deal with exchanges between cells and environment in terms of diffusion. The latter term need imply nothing of the *nature* of a barrier. Some physiologists, those of second grade, get all balled up by thinking of mechanisms for which there is little proof.

I am delighted that Chinard and Flexner are on their way to straighten out some of these matters. Indeed, much that they have presented could be put in terms of differences of chemical potentials that need imply nothing regarding the nature of barriers but that can give a rational and very simple description of facts.

Dr. Bard: I want to ask what may be a very stupid question. Of course, Starling was concerned, perhaps overly concerned, with the simple anatomical arrangement of the capillary, with the pressure relations at the arteriolar and venous ends. Now recent evidence indicates that at times in any one capillary the pressure gradient is often very small, while the actual average pressure may differ tremendously from one capillary to another. Thus, flow and pressure conditions may vary greatly in adjacent capillaries. Any consideration of the Starling hypothesis must therefore be on a statistical basis, not on the idea of what occurs in an individual capillary. But to go back to my question, is it not possible that the capillary wall may have a differential permeability as regards inulin and thiocyanate?

Dr. Chinard: It is quite possible that it may, Dr. Bard. As a matter of fact I hope that I indicated emphatically enough that actually we have to work with

tracer substances or, rather, with isotopes of naturally occurring substances. In the last analysis, final conclusions cannot be reached until one is able to analyze the results of studies on single capillaries throughout the whole body.

If one assumes what Danielli and quite a large number of others have, namely that filtration in bulk occurs through pores of such size as to permit free passage to molecules ranging from water to inulin, then there must be no separation on passage across the capillary walls of thiocyanate and inulin. Our data indicate that separation does occur. That particular assumption falls down. But the capillaries apparently distinguish between smaller substances. Dr. Flexner's earlier experiments indicate that heavy water and radioactive sodium are separated; this is in line with the concept of diffusion in both directions.

I would like to make one point about Starling, if I may. I meant to say "what appears to be the generally accepted version." I think Starling's name has been taken in vain by many modern physiologists; the interpretation put on some of his statements today he would probably deny.

Dr. Genecin: I would like to know what different results one might expect from the experiments in which the single organs were perfused by mixtures of substances of different molecular size, if we do assume that the bulk filtration and diffusion of different sized molecules occurs what would the respective curves look like and how would they differ from those demonstrated tonight?

Dr. Chinard: We have studied individual organs. There has been as little trauma as possible. We have simply given an instantaneous injection either into the main artery supplying an organ, or, where possible, a branch so that we wouldn't touch the main artery. In experiments on the kidney, a bent needle is stuck into the aorta and then turned into the opening of the renal artery to avoid touching the wall.

If bulk passage did occur in the type of experiment we have done one would expect that there would be no separation in the early portions of the curve of inulin and thiocyanate. The two would be separated from the dye curve but not from each other. With respect to the end portion of the curve, return would be by diffusion. More of the smaller, more rapidly diffusing substance would return; towards the terminal portion of the curve the concentration of the smaller substance would be higher.

Dr. Genecin: How about the glomerular capillary?

Dr. Chinard: With respect to the experiments reported here, we can't draw any conclusions; we're buffaloed by the double capillary net-work. We do know there is separation by diffusion; at least we deduce from our experiments that there is diffusion. We cannot say that it takes place at the level of the glomeruli.

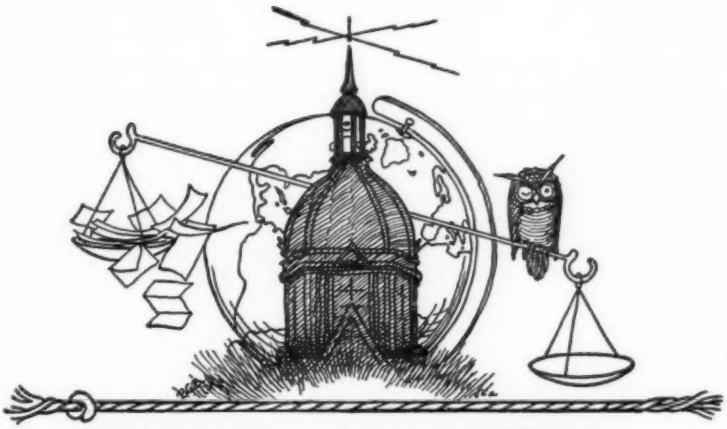
Richards demonstrated very beautifully that there was no evidence that the glomerular membrane was a secreting membrane. Concentrations of substances

studied were equal within the limits of the Gibbs-Donnan effect. I think that exception may be taken to his deduction that this equality of concentration demonstrated that diffusion cannot play a role. The period of time required for obtaining the samples was of the order of twenty minutes to an hour. He maintained the blood concentration constant during that time and the tubule was blocked off. One would expect under those circumstances that equality of concentration would be established regardless of the process involved in the passage of fluid from blood to the glomerular space. So I would say you can't tell which process is involved.

Dr. Genecin: It might be entertaining to repeat the experiments on the individual glomeruli using rapidly changing blood concentration.

Dr. Chinard: I would try to have somebody else do it.

Dr. Fisher: Are there any other questions or discussion? If not, I would like to thank the speakers and call the meeting adjourned.



BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Bulletin)

The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence. By LAWSON WILKINS. 384 pp. $13.00. *Charles C Thomas, Springfield, Illinois.*

This unique and scholarly book is appropriately dedicated to the family physician, the pediatrician, and the endocrinologist. It will prove of interest to all who have contact with the endocrinopathies. All readers, including students who have long needed such a complete and concise text, will find it a treasure of useful information carefully compiled and intelligently and attractively presented. It is bound to become a classic in its field.

The book contains twenty-one chapters each dealing with important facets of endocrine disorders and closely related fields. The chapter headings are: The Relation of Extrinsic, Genetic and Endocrine Factors to Growth and Development; Endocrine Relationships and Their Influences upon Growth and Development; Methods of Endocrine Study and Diagnosis: Clinical and Developmental Studies; Methods of Endocrine Study and Diagnosis: Tests for Hormonal Function and Assay Methods; Thyroid Disorders: I. Hypothroidism; Thyroid Disorders: II. Hyperthyroidism; Thyroid Disorders: III. Various Types of Goiter, Thyroiditis and Carcinoma of the Thyroid; Dwarfism; Variations in the Pattern of Adolescent Development; Sexual Precocity of Isosexual Pattern; Sexual Infantilism; Adrenal Cortex: I. Hormones and their Actions; Adrenal Cortex: II. Adrenal Insufficiency; Adrenal Cortex: III. Hyperadrenocorticism—Adrenogenital Syndrome and Cushing's Syndrome; Adrenal Medulla: Pheochromocytomas and Other Tumors of the Adrenal Medulla; Heterosexual Development: Pituitary Tumors and Intracranial

Lesions Causing Endocrine Disorders; Diabetes Insipidus; Calcium and Phosphorus Metabolism—Parathyroid Disorders and Tetany; Carbohydrate Metabolism and Spontaneous Hypoglycemia; Obesity; Congenital Anomalies Sometimes Mistaken for Endocrinopathies.

This material is closely integrated, yet almost never redundant. There are many valuable cross references in the text. The author speaks with the authority of long experience, yet the text is rarely didactic. It "reads easily;" and the reader quickly falls into step with the author as one who has travelled the long, sometimes trying, and always interesting road that leads to our present understanding of the many complicated conditions described.

The physical appearance of the book is unusually attractive. Numerous excellent illustrations, figures and informative tables give visual example and summation of the important points of the text. Each chapter is followed by a carefully selected and complete bibliography of key articles of historic and current review interest. Many of the chapters are enlarged and embellished by the addition of atlases of illustrations of the conditions described in the text.

The author has taken pains to describe and document carefully the classic and accepted manifestations of endocrine disease. He has however also included the unusual and difficult cases. He leaves the door open for further knowledge and advancements, yet the reader gains the impression that alterations in future editions will be in the nature of new knowledge added to an already secure foundation rather than changes in existing ideas as he has expressed them.

All important available knowledge and theory concerning each of the conditions discussed has been carefully summed up in the text. The author pays particular attention to the criteria for diagnosis, including performance and interpretation of the numerous complicated and sometimes confusing laboratory tests. He sharply outlines specific treatments where appropriate and also describes the pitfalls, uncertainties and disappointments that may exist.

The book is encyclopedic yet never ponderous. It is authoritative, clear, concise and easy to read. It should prove invaluable as a reference, text, and manual for practitioners, students and specialists.

W. E. G.

Current Therapy 1951. Latest Approved Methods of Treatment for the Practicing Physician. Edited by HOWARD F. CONN. 699 pp. $10.00. *W. B. Saunders Co., Philadelphia, Pa.*

The 1951 edition of this compendium of therapeutic regimens has added forty-nine new contributors, eighty-six new regimens and a number of the previously published regimens have been significantly changed. The volume continues to present sound therapeutic routines based on good judgment and wide experience and in many cases two or more approaches to treatment are presented for selection without comment as to the editor's preference. A sufficiently wide list of subjects is adequately covered to include the great majority of cases seen by the practitioner. The volume continues to be a valuable addition to the library of the practitioner and the medical student.

Aside from the brevity of some of the regimens there is little adverse criticism to be made of the contents of this volume. One wonders, however, if it is necessary to re-issue so much of the old material each year in order to present the important new advances which have occurred during the preceding year and whether a loose-leaf volume would not serve this purpose more effectively. As in previous years, the volume is unnecessarily large and the front cover unduly emphasizes the publisher's name in contrast to the editor's name. R. E. M.

Parasitic Infections in Man. Edited by HARRY MOST. New York Academy of Medicine Section on Microbiology. Symposium No. 4. 229 pp. $4.50. *Columbia University Press, New York.*

This symposium is a clear exposition of the life and the future that is in the study of parasites. The approach of all of the essays is dynamic. There is no looking back. The chapter on "Diagnosis of Intestinal Helminths and Protozoa: Current Perspective" by Norman Stoll, ends with the ringing challenge to other parasitologists, "The professional parasitologist, however, has a responsibility that goes even farther. His tools and techniques should be forged with an eye on the goal of the eventual parasitic defamation of man. For only in a society made up of parasite-free individuals will we know of what the *human* being is capable."

Chapters such as "The Physiology of Blood Fagellates" by van Brand; "Metabolism of Helminths" by E. Beuding, and "Biochemistry and Metabolism of Malarial Parasites" by R. W. McKee cannot be found in any of the standard books on parasitology. And yet, it is in fields such as these that the future of parasitology lies. These chapters then point the way. They show how much can be learned from a careful detailed study of the varied metabolic processes by which a malaria parasite derives its sustenance from the red cell, which it has invaded, and how much there is to be done before we can understand why an adult schistosome worm can live and survive in the relative anaerobic surroundings of the venous blood returning from the intestine.

Such a wide survey as this may become too general, as, when another author in discussing immunological mechanisms says "so important is this immune response to the ultimate eradication of malaria, that authorities usually favor permitting an infection to go on through several febrile paroxysms prior to treatment with drugs in order that the individual may develop his own immune response."

The excellence of the majority of these essays makes one only regret that it has taken two years for the symposium to be published.

F. B. B.

Techniques in British Surgery. Edited by RODNEY MAINGOT. 733 pp. $15.00. *W. B. Saunders Co., Philadelphia, Pa.*

This volume is designed to present "a liberal cross-section of British surgery as practised today." Under the editorial direction of one of Britain's foremost surgeons it is composed of a series of selected articles on surgical subjects written by twenty-nine authors, all of whom are actively engaged in the practice and teaching of surgery in England, Scotland or Wales. The presentations are grouped

under four general headings: Head, Neck and Spinal Column; Thorax; Abdomen and Pelvis; and Extremities. As implied by the title of the book the individual articles are concerned with the practical management of clinical problems with emphasis on operative technique. In each instance the clinical problem is briefly and lucidly outlined, the rationale and aims of surgical therapy are defined and the details of treatment are given with particular reference to the individual author's personal experience and results. The essays are on the whole well written and the techniques described are sound. There are numerous illustrations which amplify the accompanying descriptions of operations.

This is an informative book and a good source of reference. To the American reader it offers an opportunity for instructive comparison between present-day British and American surgical methods at the practical level. Except for minor details there appear to be many more similarities than differences.

H. W. S., Jr.

Regional Orthopedic Surgery. By Paul C. Colonna. 706 pp. $11.50. *W. B. Saunders Co., Philadelphia, Pa.*

In this text book of 700 pages which Dr. Colonna has dedicated to Dr. Royal Whitman, there is a continuous reflection of the author's broad experience as a teacher. He has succeeded in concisely covering the field of orthopedic surgery in an unique regional approach. Throughout the text, emphasis is given to diagnosis and treatment, with an anatomical review of the region under discussion and an orthopedic examination of the region, especially valuable to the student.

The introductory chapters on physiology and general pathology of bones are excellent, as a general preparation for the regional discussions. The special chapters on neuromuscular disabilities, bone tumors and physical medicine as applied to orthopedic surgery are well done and valuable adjuncts to the general text. There is an abundance of excellent illustrations, including roentgenograms, and the line-drawings are most instructive.

Dr. Colonna is to be congratulated on this very fine work and the book is highly recommended to undergraduates, graduates or to any one who has any interest in orthopedic surgery.

H. A. J.

Exotische Krankheiten und Krankheitsverläufe. By F. O. Höring. 407 pp. Ganzleinen DM 36.– ($8.55). *Georg Thieme Verlag, Stuttgart.*

This textbook of exotic infectious diseases has much to recommend it. Its classification, for the most part, is not on the basis of the etiological agent, but rather on the basis of the clinical syndrome produced. For instance, under "local infections with systemic manifestations" we find bubonic plague and myositis tropica discussed within a page of each other; elsewhere we go from trachoma to gonorrhoea (both local infections of the mucous membranes) or lymphopathia and tularemia (lymph gland infections) similarly discussed. In keeping with its clinical nature, discussions concerning the agents causing the diseases are kept to a minimum. This has one disadvantage. It is difficult to tell how one would make a

laboratory diagnosis of a particular infection. On the other hand, the many clear and yet detailed clinical charts of the course of the different infectious diseases are of great interest and help.

Naturally enough, individual chapters give varying slants. The discussion of brucellosis emphasizes the point of view that an allergic reaction to the infecting organism is the basis of much of the clinical symptomatology. Diverse treatments are listed, including the statement that "salvarsan through its stimulating effect on the reticulo-endothelial system may hasten recovery." However, the effect of aureomycin and chloromycetin on the acute phase of the disease is also noted. The therapy of lymphopathia veneria includes a long list of various agents including antimony in several forms, and the reader is given little indication as to which of these is only of historical interest. The discussion of treatment and suppression of malaria by atabrine is, as is to be expected, a full and excellent one.

This book is to be recommended to all those desiring to view infectious diseases from other lands with a clinician's vision.

F. B. B.

Muscular Contraction. A Topic in Molecular Physiology. By W. F. H. M. Mommaerts. 191 pp. $4.20. *Interscience Publishers, New York.*

The second quarter of this century has seen an explosive intensification in research aimed to disclose the ultimate structure and intimate physicochemical processes concerned in muscular contraction. The rapid rate of expansion in experimental data and theoretical treatment of observations made by widely differing technics and disciplines requires repeated review to provide distillation and interpretation of the sometimes formless and oftentimes confusing mass of information. A monograph such as this, therefore, is particularly welcome because it presents in compressed form a condensation of many of the recent studies of the energetics, and of the structure and physicochemical characteristics of certain proteins derived from muscle and related intimately to the prime muscular function, contraction. This field, recently invigorated by dramatic experiments by Szent-Györgyi and his colleagues, is replete with many isolated observations which already have deepened the attack on several fundamental mechanisms. This monograph brings together most of the scattered reports in several phases and adds other observations made by the author. As an introduction to the field this little book is to be recommended, and succeeds in large part to provide what the author intended, a monographic description of "the experimental discoveries and their immediate interpretations in a more elementary fashion."

Perhaps it is the newness of this field which underlies certain features of this book which are less satisfying. The overwhelming mass of detail precludes the extraction of general principles which do not require extensive qualification. And the defense of hypotheses occasionally employs reasonable assumptions masquerading as proved facts.

This absorbing story is not yet all told; this book is a scholarly beginning.

J. L. L., Jr.

BOOKS RECEIVED FOR REVIEW

Current Therapy 1951. Latest Approved Methods of Treatment for the Practicing Physician. Edited by HOWARD F. CONN. 699 pp. $10.00. W. B. Saunders Co., Philadelphia, Pa.

Introduction to Surgery. By VIRGINIA KNEELAND FRANTZ AND HAROLD DORTIC HARVEY. 233 pp. $2.75. Oxford University Press, New York.

Klinische Chemie und Mikroskopie. By LOTHAR HALLMANN. 594 pp. Ganzleinen DM 24.60 ($5.85). Georg Thieme Verlag, Stuttgart.

Parasitic Infections in Man. Edited by HARRY MOST. New York Academy of Medicine Section on Microbiology. Symposium No. 4. 229 pp. $4.50. Columbia University Press, New York.

Paul Ehrlich. By MARTHA MARQUARDT. 255 pp. $3.50. Henry Schuman, Inc., New York.

A Textbook of the Practice of Medicine, 8th edition. By VARIOUS AUTHORS. Edited by Frederick W. Price. 2076 pp. $9.00. Oxford University Press, New York.